RESEARCH REPORT: EYE IRRITATION AND SURFACTANT PROPERTIES OF NONIONIC SURFACTANTS

Part 2. In Vivo Test Results

February 23, 1998

Prepared for:

Non-Animal Testing Research Subcommittee Biomedical Research Committee Soap and Detergent Association 475 Park Avenue South New York, New York 10016

> John E. Heinze, Ph.D. Technical Consultant

<u>CONTENTS</u>	<u>Page</u>
EXECUTIVE SUMMARY	5
INTRODUCTION	7
Structure-Activity Relationships Among Alcohol Ethoxylates	12
CONTRACT STUDIES ON NONIONIC SURFACTANTS	20
Rationale for the New Studies	20
Selection of Nonionics for Testing	21
Selection of <u>In Vivo</u> Methods and Contract Laboratory	23
Selection of Test Methods and Contract Laboratories for Surfactant Properties	24
Surface Tension — Static	24
Surface Tension — Dynamic	25
Surface Tension — Interfacial	25
Surface Tension — Contact Angle	26
Cloud Point	26
Phase Inversion Temperature	27
Pour Point / Melting Point / Density	27
Viscosity	28
Krafft Point	28

Correlations of Surfactant Properties with Structure	28
Contract Laboratories	31
Selection of Nonionic Surfactant Doses for Testing	32
METHODS	33
Preparation of test Solutions	33
Low Volume Eye Test (LVET) Procedures	35
Surfactant Property Measurement Procedures	35
CONDEA Vista Company	35
United States Testing Company	36
SynsaDyne Instrument Division	36
TEST RESULTS	37
Preparation of Test Solutions	37
Results from the Low Volume Eye Test (LVET)	38
Descriptive Analysis	38
Statistical Analysis	41
Correlations to Physical Properties / Structure	43
Results of Equilibrium Surface Tension Measurements	48
Surfactant Effectiveness	48
Surfactant Efficiency and CMC Values	52
Results of Dynamic Surface Tension Measurements	55
Results of Interfacial Surface Tension Measurements	62

Results of Contact Angle Measurements	64				
Statistical Analysis of Surface Tension Measurements					
Surface Tension Measurement Correlations to Physical and Structural Properties	67				
рН	67				
Hydrophilic-Lipophilic Balance	67				
Alkyl Chain Length	72				
Oxyethylene (EO) Units	72				
Comparison to Predicted Correlations	77				
Surface Tension Measurement Correlations to Eye Irritation Potential	80				
CONCLUSIONS	85				
REFERENCES	89				
APPENDIX 1	91				
APPENDIX 2	92				
APPENDIX 3	93				
APPENDIX 4	94				
APPENDIX 5	95				
APPENDIX 6	96				
APPENDIX 7	97				
APPENDIX 8	98				

EXECUTIVE SUMMARY

This research report describes a program of exploratory research conducted by the Non-animal Testing Research Subcommittee of the Soap and Detergent Association on nonionic surfactants, an important class of cleaning agents used in laundry and cleaning products.

The key findings from the Subcommittee's research are:

- 1) The eye irritation potential of the nonionic surfactants, when evaluated in the low volume eye test at a concentration (0.2 M) representing the maximal use concentration in laundry and cleaning products, ranges from none to low;
- 2) For alcohol ethoxylates, eye irritation potential is predicted by the hydrophilic-lipophilic balance (HLB) with alcohol ethoxylates having HLBs lower or higher than 12 showing lower eye irritation potential than alcohol ethoxylates with HLBs of approximately 12; and
- 3) The extent to which the surfactant solution can lower the surface tension of water in 0.25 seconds or less (dynamic surface tension effectiveness at bubble rates of 4 or 10 per second) correlates to the eye irritation potential of the nonionic surfactants.

Part 1 of this report (previously issued) describes published and unpublished results from <u>in vitro</u> tests on various nonionic surfactants, concluding that there is a need for better <u>in vivo</u> eye irritation data on nonionic surfactants to provide a more reliable data base for evaluating <u>in vitro</u> test methods. Moreover, there is a need for more extensive surfactant property data on nonionic surfactants to determine if eye irritation potential can be predicted from the surfactant properties of nonionic surfactants. Part 2 of the report (this manuscript) describes the results of the Subcommittee's efforts: 1) to generate high quality <u>in vivo</u> eye irritation data and comprehensive surfactant property data on a defined set of nonionic surfactants; and 2) to conduct an in-depth analysis of this data for structure-activity relationships and for the ability of surfactant properties to predict eye irritation potential.

Part 2 begins with a review of the published and unpublished data on the eye irritation potential of nonionic surfactants. This review confirms the suspicions raised in Part 1 about the reliability of the existing in vivo eye irritation data.

Ten nonionic surfactants, including five alcohol ethoxylates, were selected for evaluation based on their common use in laundry and cleaning products and to test for structure-activity relationships. The five alcohol ethoxylates were (where the numbers following "A" indicates the alcohol chain length and the number following "EO" indicates the average number of oxyethylene units): A_{12} - $_{13}EO_{6.5}$, $A_{12-14}EO_7$, $A_{8-10}EO_5$, $A_{12-13}EO_3$ and $A_{12}EO_{23}$. The other five surfactants were: nonylphenol-EO₉, sorbitan trioleate-EO₂₀, A_{12-16} - glucose_{1.6}, lauramine oxide and cocamide diethanol amine.

The surfactants were evaluated in aqueous solutions at the same molar concentration (0.2 M) to facilitate comparisons between eye irritation potential and surfactant properties. The surfactant concentration was chosen to represent the maximum use concentration in aqueous laundry and cleaning products.

The low volume eye test (LVET) was selected as the <u>in vivo</u> method to evaluate eye irritation potential because it is uses fewer animals than the standard Draize eye test and because of the favorable experience with this test method in previous research sponsored by the Subcommittee.

The following surfactant properties were chosen for evaluation: equilibrium and dynamic surface tension "effectiveness" (surface tension at or above the critical micelle concentration or CMC), "CMC" (maximal surfactant concentration in water before formation of micelle aggregates), and "efficiency" (surfactant concentration to reduce the surface tension of water by 20 dynes/cm), "interfacial tension" (the surface tension at a water-organic liquid (octanol) interface) and "contact angle" (the angle formed by a drop of an aqueous solution on a smooth, flat surface (TEFLON tape). These are fundamental properties of all nonionic surfactants.

Member company and contract laboratories to preform the testing (CONDEA Vista Company, Hazleton Wisconsin, SynsaDyne Instrument Division, and United States Testing Corporation) were selected based on their expertise and experience in conducting the evaluations.

The maximum average scores (MAS) and medium days to clear (MDTC) in the LVET were determined for eleven surfactant solutions. MAS values ranged from 0.0 to 13.3 (on a scale of 0 to 110) and the MDTC from 0 to 4 days. Based on these scores, the eye irritation potential of the surfactant solutions can be considered to range from none to low. Despite the narrow range of MAS scores, the difference among the surfactant solutions was highly significant (p<0.001) based on analysis of variance (ANOVA).

For the alcohol ethoxylates among the nonionic surfactants tested, HLB values predict the relative eye irritation potential, with higher MAS scores for the three alcohol ethoxylates with HLBs of approximately 12 and lower MAS scores for the two alcohol ethoxylates with either a lower or a higher HLB.

Based on regression analysis, there were no significant correlations between MAS scores and any equilibrium surface tension measurement. However, significant correlations to MAS scores were found for surface tension effectiveness measured under two dynamic conditions.

This is the first report of a significant correlation between eye irritation potential and any surfactant property. Dynamic surface tension may be predictive of the eye irritation potential of nonionic surfactants at the concentrations used in laundry and cleaning products.

INTRODUCTION

Part 1 of this report ("<u>In Vitro</u> Test Results") describes the background to the research on nonionic surfactants conducted by the Non-animal Testing Research Subcommittee of the Soap and Detergent Association (SDA). This research is a continuation of previous SDA efforts to evaluate the ability of <u>in vitro</u> tests to predict the eye irritation potential of cleaning products and ingredients.

The specific goal of Part 1 of the research on nonionic surfactants was to determine if the eye irritation potential of nonionic surfactants, an important class of ingredients used in detergents and cleaning products, can be predicted by <u>in vitro</u> tests. The findings of a literature review, and especially the results of unpublished data made available to the SDA, suggest not. In fact, all eight of the <u>in vitro</u> assays examined misclassified (false positive or false negative response) one or more of the nonionics examined (Tables 1 - 2).

Surfactant Brand name/ abbreviation	3T3 mouse cells Uridine uptake 4 hr. exposure	SIRC corneal cells Colony formation 1 hr. exposure	MICROTOX LBT Light emission 15 minute exposure	SKIN ² ZK 1200 MTT uptake 30 minute exposure	SKIN ² ZK1200 MTT uptake Topical exposure
SLS					FN
TRITON X-100					
TRITON X-155				FN	FN
GLUCOPON 625CS		FP	FP		
BRIJ-35	FP	FP			
TWEEN 20					
TWEEN 40					
TWEEN 80					

 Table 1

 Summary of False Negative/False Positive Responses Observed with In Vitro Tests¹

¹ See Tables 1, 2 and 3 in Part 1 of this report for details. "--" = not tested; "FP" = false positive response; "FN" = false negative response.

Structure-activity relationships among the nonionic surfactants were also examined in Part 1 to determine if the reported eye irritation potential or in vitro test results could be correlated to

surfactant properties of the nonionics. No relationship to either <u>in vivo</u> or <u>in vitro</u> results were apparent for the surfactant properties examined (surface tension, critical micelle concentration, octanol-water partitioning or hydrophilic-lipophilic balance). However, there were a number of limitations in the data on surfactant properties which may have confounded the search for structureactivity relationships.

Summary 0	Summary of False Negative/Positive Responses, irreproducionity, with min view resis					
Surfactant Brand name/ abbreviation	SIRC corneal cells Colony formation 1 hr. exposure	MICROTOX LBT Light emission 15 minute exposure	Skin ² ZK1200 Neutral red uptake 4 hours exposure	Skin ² ZK1200 PGE2 release 4 hours exposure ²	CAMVA hen egg Vascular changes	
SLS						
AE 810 (50:50)-7.8	FN		FN		?3	
AE 1214 (50:50)-6.9	FP	FP	FP	FP	?3	
GLUCOPON 625CS	(FP)	(FP)			FP	
BRIJ-35	(FP)					
AE 1214 (50:50)-1.1	FP	FP				

Table 2	
ummany of False Negative/Positive Responses.	Irreproducibility, with In Vitro Tests ¹

¹ See Tables 4, 5 and 7 in Part 1 of this report for details. "--" = not tested; "FP" = false positive response; "FN" = false negative response; "()" = same as Table 1.

²Results in this assay were not reproducible. See Table 6 in Part 1.

³ Either the result with AE 810(50:50)-7.8 is a false negative response or that with AE 1214(50:50)-6.9 is a false positive response.

The results described in Part 1 also raise questions about the quality of the <u>in vivo</u> eye irritation data available on nonionic surfactants. For instance, the evaluations in Table 1 depend on the accuracy of the classification of the eye irritation potential of the nine surfactants. The classification was derived from three sources: unpublished data from Henkel Corporation for GLUCOPON 625CS, published Draize eye test results for three surfactants (Kennah et al., 1989), and a published compilation of data (Grant, 1989) for six surfactants.

The Henkel data on GLUCOPON 625CS indicates that a 15% (active) aqueous solution of a structurally similar alkylpolyglucoside ("APG 550") gave a maximum average Draize score of 4 (on a scale of 0 to 110, where increasing scores indicate increasing severity of irritation). In comparison,

a 15% active solution of a commonly used anionic surfactant, sodium laureth (3 oxyethylene units) sulfate, is reported to give maximum average scores of 30 to 35. This data suggests that GLUCOPON 625CS should be classified as "mild" as claimed by Henkel. However, this classification is based on an unpublished study conducted on a material similar, but not identical, to GLUCOPON 625CS. Moreover, the Material Safety Data Sheet on GLUCOPON 625CS (included in Appendix 2) reports that, when the 50% active product was tested in six animals, the maximum score in each animal ranged from 39 to 61, suggesting that the undiluted product should be classified as a moderate irritant.

The data of Kennah et al. (1989) on sodium lauryl sulfate (SLS), TRITON X-100, and TWEEN 20 is from a published report which includes the actual Draize test maximum average score (MAS) on each compound (Table 3). Compounds were tested at three concentrations covering a range of those used in detergents and cleaning products. The coefficient of variation of the MAS values is also given to indicate the range of MAS values observed in each test. The data available on the eye irritation potential of these three materials appears to be of high quality.

I ublished	I ublished Diale Lye Articlea Data of Land						
Surfactant brand name/ abbreviation ²	Concentration (volume %)	Draize Score (MAS) ³	Coefficient of Variation (%) ⁴	Eye Irritation Classification			
SLS	30	61	8.3	Severe			
	15	59	5.7				
	3	16	19				
TRITON X-100	10	59	2.0	Severe			
	5	32	18				
	1	2	59				
TWEEN 20	10	1	86	Mild			
	5	1	86				
	1	0					

Table 3Published Draize Eye Irritation Data on Three Surfactants1

¹ Data of Kennah et al., 1989.

 3 MAS = maximum average score (0 to 110 scale).

² See Table 1 in Part 1 for full names.

 4 CV = (standard deviation / mean) x 100.

The data on the six surfactants from the compilation of Grant (1989) range in quality. For instance, there is considerable data cited to support the classification of TWEEN 80 as "mild." This data includes: 1) a report by Draize and Kelly (1952) that the maximal concentration allowing any corneal or iris lesions to heal by the seventh day (the "maximum tolerated concentration") for TWEEN 80 is 100%; 2) a summary report by L. W. Hazleton (1952) indicating that TWEEN 80 produced no irritation or opacity in rabbits eyes when tested undiluted; 3) a later report by J. F. Treon (1965) confirming that "polysorbate 80," (the Cosmetic Toiletry and Fragrance Association, Inc., name for TWEEN 80) was nonirritating in rabbit eyes; and 4) a report by Hagiwara and Sugiwia (1953) that U.S. Pharmacopia grade polysorbate 80 is "well tolerated" by human subjects when applied in concentrations up to 20% in aqueous solution.

The data on the mildness of TWEEN 20 is somewhat less extensive, but is still based on three sets of data: 1) a report of comparable mildness to TWEEN 80 in the studies by Draize and Kelly (1952); 2) a report that undiluted TWEEN 20 was nonirritating and produced only "extremely mild and transient opacity" in rabbit eyes (Hazleton, 1952); and 3) the more recent report of Kennah et al (1989), reviewed above (Table 3).

The data on the mildness of BRIJ-35 is also less extensive, but no less convincing, consisting of: 1) the report by Hazleton (1952) that the undiluted surfactant is nonirritating and produces no opacity in rabbit eyes; and 2) the more recent report by North-Root et al. (1982) that the material is considered nonirritating based on an observed maximum average Draize score for the undiluted material (97% active) of 10.2 (0 to 110 scale).

The data on the mildness of TWEEN 40 is even less extensive, consisting of: 1) the report by Hazleton (1952) that the undiluted surfactant is nonirritating and produces no opacity in rabbit eyes; and 2) a more recent report by North-Root et al. (1982) that a greater than 90% concentration of the material would be required to produce a maximum average Draize score of 20. The latter estimate is based on testing a 30% solution of the material by direct corneal application of 0.01 ml to one eye of three rabbits (the low volume eye test, (Griffith et al., 1980).

The data on the eye irritation potential of TRITON X-155 is based on a single report that a "1% concentration applied four times within 25 minutes to a human eye caused pain, photophobia and loss of corneal epithelium, but the eye healed rapidly" (Grant, 1986, page 873). The citation for this report is a study by Feldman et al. (1948) on the use of various surfactants in combination with ophthalmic drugs. In this study, Feldman instilled three drops of a 1% aqueous solution in one eye of a rabbit and observed the eyes immediately after instillation and at half hour intervals over a three hour period. He also examined the eyes the next morning and repeated the dose twice over a three hour period. Feldman et al. report (page 670) that "TRITON X-135" and ULTRAWET 30 DS (a sodium alkylbenzene sulfonate) gave no visible reaction at any time. Later references to these surfactants (pages 671 and following) make it clear that the nonionic surfactant was actually TRITON X-155.

Further studies cited in this same reference (Feldman et al., 1948, page 671) indicate that no ill effects were note in 50 patients who received drops of either surfactant solution in one or both eyes. The adverse reaction noted by Grant (1986) was observed in only one of 57 patients with ophthalmic disorders who received four drops of a pupil dilator (0.5% paredrine hydrobromide) in combination with 1% TRITON X-155. No ill effects were reported for the other 56 patients receiving this combination. One of 19 patients receiving an anaesthetic (0.5% tetracaine hydrochloride) in combination with 1% TRITON X-155 experienced an intense burning sensation and heavy mucoid discharge. Five patients using pilocarpine hydrochloride (0.5% in combination with TRITON X-155, ULTRAWET 30 DS or TWEEN 80) at home for treatment of glaucoma developed pain so severe that use of the drug at to be discontinued. Three other patients used this drug in combination with TRITON X-155 for three months with no ill effects. No ill effects were reported for the other 185 patients treated with various drugs in combination with 1% TRITON X-155.

In short, Feldman et al. (1948) report that 1% TRITON X-155 is not a rabbit or human eye irritant but some patents treated with combinations of TRITON X-155 and various ophthalmic drugs may experience eye irritation. Consequently, the evidence that TRITON X-155 is an eye irritant is rather weak.

The data on the eye irritation potential of the alcohol ethoxylates in Table 2 is the only results actually generated on the identical compounds to those examined in the <u>in vitro</u> tests. All three alcohol ethoxylates were tested in the standard Draize test at the same time and by the same laboratory. Consequently, the maximum average Draize scores and relative eye irritation potentials can be reliably compared. However, only undiluted materials were tested so there is no information on the eye irritation potential of aqueous solutions of these compounds.

Benke et al. (1977) reported results with undiluted and aqueous solutions of two alcohol ethoxylates typical of those used in laundry detergents and similar to AE 1214(50:50)-6.9 in Table 2. The two alcohol ethoxylates tested were a C12-C13 AE with 6.5 oxyethylene units where the distribution of alkyl chains was 42% C-12 and 58% C-13 (AE 1213(42:58)-6.5) and a C14-C15 AE with 7.0 oxyethylene units where the alkyl chain distribution was 58% C-14 and 42% C-15 (AE 1415(58:42)-7.0). Both materials were reported to be severe eye irritants when tested undiluted in the standard Draize test. The maximum average scores ranged from 54 to 60, and many of the treated eyes did not clear within 35 days. Consequently, both of these alcohol ethoxylates were more irritating to rabbit eyes than AE 1214(50:50)-6.9.

When 10% dilutions of the two alcohol ethoxylates were tested (Benke et al. 1977), moderate, reversible irritation was produced with maximum average scores ranging from 10 to 35. These results are similar to that observed with undiluted AE 1214(50:50)-6.9.

The results with the 10% alcohol ethoxylate solutions are similar to that reported in the SDA Phase III testing on a 10% aqueous solution of an alcohol ethoxylate (Bagley et al., 1994). In the SDA test the maximum average score was 14.7 and the median days to clear was 4. The actual material tested

is not fully described in Bagley et al. (1994), but was probably a CONDEA Vista C12-C14 alcohol ethoxylate with 7.0 oxyethylene units where the alkyl chain distribution was 20% C12 and 80% C-14 (AE 1214(20:80)-7.0) (McCormick, 1993). Consequently, this material is similar to the two alcohol ethoxylates tested by Benke et al. (1977) and very similar to AE 1214(50:50)-6.9 in Table 2. Nonetheless, the published data on alcohol ethoxylates in Benke et al. (1977) are not consistent with the relatively mild eye irritation potential reported for AE 1214(50:50)-6.9 in Table 2.

In short, a review of the published and unpublished data used to support the eye irritation classification of the nonionics tested in Part 1 of this report reveals: 1) the mildness classification of some nonionics, e.g. GLUCOPON 625CS, depends on the concentration tested while other nonionics, e.g. TWEEN 20, are mild when tested undiluted or diluted; 2) the irritancy of some nonionics, e.g. TRITON X-100, is observed when tested diluted but the eye irritation potentials of other nonionics, e.g., all the alcohol ethoxylates except for AE 1214-7, have only been tested on the undiluted materials; 3) there is considerable variation in the quality and quantity of the published data used to support the eye irritation classification of nonionics, e.g. TRITON X-100 versus TRITON X-155, and 4) there are inconsistencies between the published and unpublished data on the mildness of one alcohol ethoxylate, AE 1214(50:50)-6.9.

Unfortunately, eliminating the nonionics for which there is some question about the <u>in vivo</u> data (GLUCOPON 625CS, all alcohol ethoxylates, TRITON X-155) eliminates most of the nonionics tested but not all of the false positive/false negative results observed with <u>in vitro</u> tests. See Tables 4 and 5 on the next page.

Structure-Activity Relationships Among Alcohol Ethoxylates

In an attempt to better understand the eye irritation potential of alcohol ethoxylates, all available eye irritation data on this class of nonionic surfactants was solicited from member companies. Data, consisting of summaries of unpublished studies, were provided by CONDEA Vista, Procter & Gamble and Shell Oil. The summary reports are collected in Appendix 1.

The first set of data to be analyzed was a sub-set of the CONDEA Vista data consisting of seven laboratory-prepared alcohol ethoxylates recently tested in a contract laboratory using the standard Draize test procedure (Table 6). These compounds are typical of ALFONIC-brand alcohol ethoxylates although they are not identical to any commercial product. It was felt that any structure-activity relationship would be most apparent with this set since a number of potentially confounding factors for structure-activity relationship (differences in products between manufacturers and over time, differences in test methods between laboratories and over time, etc.) are not applicable with this set.

Kevised 3	Summary of rai	se negative/r als	e i usitive nesp	onses with <u>m</u>	ILLO I COLO
Surfactant Brand name/ abbreviation	3T3 mouse cells Uridine uptake 4 hr. exposure	SIRC corneal cells Colony formation 1 hr. exposure	MICROTOX LBT Light emission 15 minute exposure	SKIN ² ZK1200 MTT uptake 30 minute exposure	SKIN ² ZK1200 MTT uptake Topical exposure
SLS					FN
TRITON X-100					
BRIJ-35	FP	FP			
TWEEN 20					
TWEEN 40					
TWEEN 80					

Table 4
 Table 4
 Revised Summary of False Negative/False Positive Responses with <u>In Vitro</u> Tests¹

¹ Revision of Table 1 in which the results with TRITON X-155 and GLUCOPON 625CS have been deleted due to uncertainties regarding their eye irritation potential. "--" = not tested; "FP" = false positive response; "FN" = false negative response.

Surfactant Brand name/ abbreviation	SIRC corneal cells Colony formation 1 hr. exposure	MICROTOX LBT Light emission 15 minute exposure	Skin ² ZK1200 Neutral red uptake 4 hours exposure	Skin ² ZK1200 PGE2 release 4 hours exposure ²	CAMVA hen egg Vascular changes
SLS					
BRIJ-35	(FP)				

Table 5Revised Summary of False Negative/Positive Responses with In Vitro Tests1

¹ Revision of Table 2 in which the results with the alcohol ethoxylates and GLUCOPON 625CS have been deleted due to uncertainties regarding their eye irritation potential. "(FP)" = false positive response (same as Table 4). ² Results in this assay were not reproducible. See Table 6 in Part 1.

An examination of the data in Table 6 indicates that there is no apparent relationship between MAS and alcohol chain length. However, there is some indication of a relationship between MAS and polyoxyethylene (EO) content whether expressed as moles of EO or weight percent. It should be

Draize Eye Test Data on Alconor Ethoxylates Tested by COT(2211 + 25th						
Alcohol Ethoxylate ¹	MAS ²	Alcohol Chain Length ³	Moles EO ⁴	Log MAS	Log EO	Log %EO
810EE-40	27.7	9.0	2.1	1.4425	0.3222	1.6021
810EE-70 ⁵	33.8	9.0	7.8	1.5289	0.8921	1.8451
1214EE-20 ⁵	4.3	13.0	1.1	0.6335	0.0414	1.3010
1214EE-60 ⁵	19.7	13.0	6.9	1.2945	0.8388	1.7782
1214EE-70	43.5	13.0	10.7	1.6385	1.0295	1.8451
1618EE-60	16.8	17.0	8.7	1.2253	0.9395	1.7782
1618EE-70	29.3	17.0	13.7	1.4669	1.1367	1.8451
	Draize Eye Alcohol Ethoxylate ¹ 810EE-40 810EE-70 ⁵ 1214EE-20 ⁵ 1214EE-60 ⁵ 1214EE-70 1618EE-60 1618EE-70	Alcohol Ethoxylate ¹ MAS ² 810EE-40 27.7 810EE-70 ⁵ 33.8 1214EE-20 ⁵ 4.3 1214EE-60 ⁵ 19.7 1214EE-70 43.5 1618EE-60 16.8 1618EE-70 29.3	Alcohol Ethoxylate ¹ MAS ² Alcohol Chain Length ³ 810EE-40 27.7 9.0 810EE-70 ⁵ 33.8 9.0 1214EE-20 ⁵ 4.3 13.0 1214EE-60 ⁵ 19.7 13.0 1214EE-70 43.5 13.0 1618EE-60 16.8 17.0	Draize Eye Test Data off Arconol Echocytate Alcohol Ethoxylate ¹ MAS ² Alcohol Chain Length ³ Moles EO ⁴ 810EE-40 27.7 9.0 2.1 810EE-70 ⁵ 33.8 9.0 7.8 1214EE-20 ⁵ 4.3 13.0 1.1 1214EE-60 ⁵ 19.7 13.0 6.9 1214EE-70 43.5 13.0 10.7 1618EE-60 16.8 17.0 8.7 1618EE-70 29.3 17.0 13.7	Draize Eyerest Data on Arconol Ethoxytates Tested 5Alcohol Ethoxylate1MAS2Alcohol Chain Length3Moles EO4Log MAS810EE-4027.79.02.11.4425810EE-70533.89.07.81.52891214EE-2054.313.01.10.63351214EE-60519.713.06.91.29451214EE-7043.513.010.71.63851618EE-6016.817.08.71.22531618EE-7029.317.013.71.4669	Draize Eyerest Data on Arconol Etnoxytates Tested by CorresAlcohol Ethoxylate1MAS2Alcohol Chain Length3Moles EO4Log MASLog EO810EE-4027.79.02.11.44250.3222810EE-70533.89.07.81.52890.89211214EE-2054.313.01.10.63350.04141214EE-60519.713.06.91.29450.83881214EE-7043.513.010.71.63851.02951618EE-6016.817.08.71.22530.93951618EE-7029.317.013.71.46691.1367

 Table 6

 Draize Eve Test Data on Alcohol Ethoxylates Tested by CONDEA Vista

¹ 1992 CONDEA Vista nomenclature, where the first pair of numbers indicate the range of alcohol chain lengths, the letters indicate the approximate weight percent of the alcohols (where A = 10%, B = 20%, etc.) and the number following the hyphen indicates the weight of the oxyethylene chain as a percent of the total molecule. Thus, "810EE-40" is predominately composed of C-8 and C-10 alcohols, the alcohols are in the ratio of 50:50, and the ethoxylate chain is 40% of the total weight of the molecule.

 2 MAS = maximum average score in standard Draize eye test.

³ Average carbon chain length.

 $^{4}EO = oxyethylene units per molecule.$

⁵ Alcohol ethoxylates tested in Part 1 of this report, Tables 4, 5 and 7.

noted that weight percent EO is the ratio of the polyoxyethylene content to the alcohol chain content and, as discussed in Part 1 of this report, is directly related to the hydrophilic-lipophilic balance (HLB) by the equation: (weight % EO) / 5 = HLB.

The relationship between the maximum average Draize scores and the EO content of these alcohol ethoxylates was explored using a linear regression program (STAT-PACKETS). This program automatically determines the correlation coefficient (R^2 -value) for various transformations of the data. Correlation coefficients are a measure of the amount of variation accounted for by the linear regression on a scale of 0 (no correlation) to 1 (100% correlation). Thus the value of correlation coefficient provides an indication of the goodness of fit of the data to the regression line.

The STAT PACKETS program also calculates analysis of valiance (ANOVA), a measure of the association between two or more variables. Unlike linear regression, ANOVA does not assume a linear relationship between variables. The most useful single statistic from ANOVA is the p-value, the estimate of the probability that the association between the variables is due to chance. P-values less than 0.05 are generally considered unlikely to be due to chance and thus are "significant."

A p-value can also be calculated from the correlation coefficient using the "t-test." For this calculation, t = square root {DF x $R^2 / (1-R^2)$ }, where "DF" is the degrees of freedom and R^2 is the correlation coefficient. For any line, DF = n - 2, where "n" is the number of data points for the line. P-values can be determined from a table of t-values for n - 2 degrees of freedom.

The statistical analysis of the relationship between the maximum average Draize scores and the EO content for the seven CONDEA Vista alcohol ethoxylates tested at the same time are summarized in Table 7.

••					¥
	Data Trans- formation	R-squared value Wt. % EO ²	R-squared value Moles EO ³	P-value⁴ Wt. % EO	P-value⁴ Moles EO
	None	0.56	0.34	0.06	5
	Log-log	0.766	0.54	0.007	5

 Table 7

 Statistical Analysis: Draize MAS vs. EO Content, Seven Alcohol Ethoxylates¹

¹ Statistical analysis of the relationship between the maximum average score (MAS) and the oxyethylene units (EO) of the alcohol ethoxylates in Table 6. Analysis using the STAT PACKETS computer program.

² Correlation coefficient (R-squared value) determined from regression analysis based on the weight percent EO.

³ Correlation coefficient (R-squared value) determined from regression analysis based on the moles of EO.

⁴ANOVA probability that association between MAS and EO content is due to chance alone.

⁵ Analysis of variance requires at least one replicate value. Consequently, ANOVA could not be performed with moles of EO as the independent variable since there were no replicate values.

⁶ T (calculated as described in text) = 4.01, DF = 5, p = 0.01.

The results of this analysis confirm an association between the maximum average Draize score and the EO content of the alcohol ethoxylates. Comparison of the correlation coefficients indicates a better correlation of MAS with the weight percent oxyethylene units than with the moles of EO. The log-log transformation, in which both the MAS and the EO content values are converted to the base-10 logarithms, also improved the correlation, increasing R²-values. Indeed, the R²-value for the log-log transformed data on weight percent EO is highly significant (p = 0.01). Moreover, log-log transformation also improved the p-value from the ANOVA for weight percent EO, reducing the probability that the association is due to chance from a marginally significant value (0.06) to a highly significant value (<0.01).

Other procedures for transformation of data, for instance the double-reciprocal transformation, also increased the correlation coefficients and reduced p-values. However, the log transformation is commonly used with biological data because the variation in the raw data tends to increase proportionateley to the mean values. Log transformation equalizes the variation and allows a more powerful statistical analysis.

Consequently, the log-log transformation of the MAS versus weight percent EO data was used in this analysis. A plot of log MAS versus log weight percent EO for the seven CONDEA Vista alcohol ethoxylates is shown in Figure 1 below.



As shown in Table 8, analysis of the entire CONDEA Vista data set, which consists of 28 alcohol ethoxylates, decreased the correlation coefficient slightly but increased the significance by ANOVA. This data set contains results from compounds tested at different laboratories over a 20+ year period. Differences in experimental procedures between laboratories and over time are the likely explanation for the decrease in the R²-value compared to the results with the alcohol ethoxylates tested at one time in the same laboratory (Tables 6 and 7). Nonetheless, the fit of the entire data set to the regression line appears to be quite good (Figure 2).

Also as shown in Table 8, addition of the Procter & Gamble and Shell Oil data on alcohol ethoxylates leads to a substantial reduction in the correlation coefficient and an increase in the p-value (despite nearly doubling the sample size). This result indicates that pooling the data from the three companies has increased the variability in the data. Additional sources of variability may include differences in the alcohol ethoxylates tested by the three companies, as well as differences in experimental procedures between laboratories and over time.

As illustrated in Figure 3, one notable difference between the alcohol ethylates tested by the three companies is the range of weight percent EO. The alcohol ethoxylates tested by CONDEA Vista ranged from 20 to 70% EO, those tested by Procter & Gamble ranged from 46 to 84% and those

Source of Data	Number of samples	R-squared value Wt. % EO ²	P-value ³ Wt. % EO
CONDEA Vista⁴	7	0.76	0.007
CONDEA Vista ⁵	28	0.66	<0.0001
All ⁶	48	0.35	0.0006

 Table 8

 Statistical Analysis: Draize MAS vs. EO Content of Alcohol Ethoxylates¹

¹ Statistical analysis of the relationship between the maximum average score (MAS) and the oxyethylene units (EO) of alcohol ethoxylates in the Draize eye test. Analysis using the STAT PACKETS computer program after log-log transformation of the data.

² Correlation coefficient (R-squared value) determined from regression analysis based on the weight percent EO.

³ ANOVA probability that association between MAS and EO content is due to chance alone.

⁴ Data and analysis from Tables 5 & 6.

⁵ Includes data on the alcohol ethoxylates in Table 5 and 21 others. See Appendix 1 for details on data set.

⁶ Includes data from CONDEA Vista, Procter & Gamble Company and Shell Oil Company. See Appendix 1 for details.



tested by Shell Oil from 40 to 62% (See Appendix 1 for calculations). Note that the untransformed data is plotted in Figure 3 because transformation did not improve the R^2 or p-values of the Procter or Shell data when analyzed separately from the CONDEA Vista data.

The regression lines fit to the untransformed data from the individual companies show different slopes. The CONDEA Vista data shows a positive slope (b = +1.1), apparently due to the low irritation potential of the low weight percent (EO < 40%) alcohol ethoxylates. In contrast, the Procter & Gamble data shows a negative slope (b = -0.55), apparently due to the low irritation potential of the high weight percent (EO > 80%) alcohol ethoxylates. The Shell Oil data, which contains intermediate weight percent ethoxylates, shows an intermediate, slightly positive slope (b = +0.37). This analysis suggests that the true shape of the curve for weight percent EO versus MAS for the combined data set (Figure 3) may be bell-shaped, with the maximum irritation at about 60 weight percent EO and lower irritation at lower and higher weight percent EO. However, additional alcohol ethoxylates with low, middle and high weight percent EOs would have to be tested simultaneously at the same lab, and using the same test procedure, to confirm this structure-activity relationship.



Low eye irritation potential of the low weight percent EO alcohol ethoxylates may be due to the higher content of un-ethoxylated (free) alcohols. As shown in Table 9, the free alcohol content of alcohol ethoxylates is higher at lower levels of ethoxylation. However, unethoxylated alcohols have appreciable eye irritation potential as shown in Figure 4. In fact, the eye irritation potential of the free alcohol entirely accounts for the eye irritation observed for the C12-C14 alcohol ethoxylates in Table 9. Consequently, the low eye irritation potential of the low weight percent EO alcohol ethoxylates appears to be due to the alcohol ethoxylates themselves.

		Tat	ole 9		
Lack of Co	rrelation of Fre	e Alcohol Conte	ent to Draize M	AS for Alcohol	Ethoxylates

Alcohol Ethoxylate ^{1,2}	Free alcohol content (%) ²	Alcohol chain length (average) ²	MAS of free alcohol ³	Calculated MAS due to alcohol content ⁴	Observed MAS of alcohol ethoxylate ²
1214EE-20	33.3	13.0	24.0	8.0	4.3
1214HA-30	15.0	12.3	28.1	4.2	3.0
1412HA-30	13.5	13.7	19.9	2.7	3.0
810EE-40	12.2	9.0	47.4	5.8	27.7

¹ CONDEA Vista low weight percent EO alcohol ethoxylates (free alcohol >10%). See Table 6, note 1, for details on the nomenclature.

² Data from Appendix 1.

³ Calculated from alcohol chain length (average) using the regression equation from Figure 4: MAS = (-5.86)(alcohol chain length) + 100.

⁴ Free alcohol content x MAS of free alcohol.



To recap, the CONDEA Vista data, especially after log-log transformation, shows that the lower weight percent EO alcohol ethoxylates (EO < 40%) are milder than the mid-range alcohol ethoxylates (i.e. about 60% EO). Mildness is due to the alcohol ethoxylates themselves, not the higher free alcohol content of the lower weight percent ethoxylates. The combined data from

CONDEA Vista, Procter & Gamble and Shell Oil shows more variability than the CONDEA Vista data alone. Greater variability is at least partly due to the different ranges of EO content tested by the three companies. There is some indication from the Procter & Gamble data that higher weight percent EO alcohol ethoxylates (EO > 80%) are also milder than mid-range alcohol ethoxylates, suggesting that the structure-activity curve for maximum average Draize score versus weight percent EO is bell-shaped. However, this would need to be confirmed by testing a set of alcohol ethoxylates differing only in their weight percent EO at one time in the same laboratory to eliminate other possible sources of variation in the analysis.

CONTRACT STUDIES ON NONIONIC SURFACTANTS

Up to this point, this report has reviewed the test results and analysis of historic data generated by member companies. Now the report will focus on those studies conducted on nonionic surfactants by the Non-animal Testing subcommittee under contract to various laboratories. These studies were intended to resolve some of the questions raised by the previous research on nonionic surfactants.

Rationale for the New Studies

Contract studies were based on the following conclusions from the previous research:

1) The eye irritation potential of nonionic surfactants is not well predicted by the various <u>in</u> <u>vitro</u> tests that have been examined. However, there is some uncertainty in this conclusion because of deficiencies in the <u>in vivo</u> data used to classify the eye irritation potential of the nonionics. Consequently, there was a need for high quality <u>in vivo</u> data on a set of nonionic surfactants used in laundry and cleaning products. This set, once generated, could be used to evaluate the ability of <u>in vitro</u> tests to predict their eye irritation potential.

2) A major difficulty with some of the <u>in vivo</u> data is that it is based on testing undiluted nonionics. Results from such testing may not be relevant to the eye irritation potential in laundry and cleaning products where lower concentrations are likely to be used. This is particularly true for alcohol ethoxylates which are supplied as 100% active stocks and are essentially nonaqueous liquids or solids. The eye irritation potential of aqueous solutions of such nonionics are likely to be very different from that of the 100% active stocks. Consequently, there was a need for eye irritation data on nonionics tested in aqueous solution at concentrations representative of in-use concentrations of laundry and cleaning products.

3) Surfactant properties of nonionics may be useful in predicting their eye irritation potential. However, attempts to determine structure-activity relationships among the nonionic surfactants used to evaluate the <u>in vitro</u> tests were unsuccessful, probably due to

the lack of surfactant property data on the nonionics. Consequently, there was a need for a complete set of surfactant property data on the same set of laundry and cleaning product nonionics to be tested for eye irritation potential.

Selection of Nonionics for Testing

The nonionics selected by the Subcommittee for testing are shown in Table 10. Shown are the Subcommittee's names for the test materials as well as the brand names and suppliers. Also shown are the percent actives and the HLB values provided by the suppliers (see Appendix 2). Calculation of the HLBs for cocamide DEA and lauramine oxide is based on the method used by Henkel (Smith, 1997) as shown in Figure 5. It should be noted that this method of calculation is mathematically equivalent to that used previously for alcohol ethoxylates (HLB = [weight % EO] / 5) since the oxyethylene (EO) units are the hydrophile in alcohol ethoxylates and weight % EO = 100 x (molecular weight hydrophile) / total molecular weight.

The chemical formulas shown in Table 10 are based on supplier information or structural formulas (Appendix 2) shown in the Cosmetic Toiletry and Fragrance Association (CTFA) *Cosmetic Ingredient Dictionary* (CTFA, 1977). CTFA names for the test materials are indicated in the footnotes to Table 10. Note that CTFA names are not available for the first four alcohol ethoxylates because these materials are primarily used in laundry detergents and cleaning products.

Also shown in Table 10 are the average molecular weights of the test materials based on the chemical formulas. The percent actives and molecular weights are needed to calculate the weight of material for preparation of the stock solutions (see below).

The Subcommittee's rationale for the selection of these nonionics for testing is as follows. First, all materials had to be nonionics commonly used in the detergent and cleaning products industry.

The five alcohol ethoxylates tested were specifically selected to examine the hypothesis developed in the previous section ("Structure-Activity Relationships Among Alcohol Ethoxylates") that maximum eye irritation potential seems to occur with mid-range weight percent (about 60% oxyethylene [EO] units, HLB = approximately 12) alcohol ethoxylates with both lower (EO < 40%, HLB < 8) and higher (EO > 80%, HLB > 16) weight percent alcohol ethoxylates having lower eye irritation potential. Consequently, the first three alcohol ethoxylates (A_{12-13} -EO_{6.5}, A_{12-14} -EO₇, A_{8-10} -EO₅) have virtually identical HLBs but differ in their alcohol and EO chain lengths while the forth (A_{12-13} -EO₃) and fifth (A_{12} -EO₂₃) alcohol ethoxylates have either lower or higher HLBs but similar alcohol chain lengths to the first alcohol ethoxylate. It should be noted that the first two alcohol ethoxylates listed in Table 10 are typical of those used in laundry detergents.

The next three compounds after the alcohol ethoxylates in Table 10 (nonylphenol-EO₉, sorbitan trioleate-EO₂₀ and A_{12-16} -glucose_{1.6}) were selected on the basis of having HLB values similar to those

of the first three alcohol ethoxylates but differing in chemical structure. Specifically, nonylphenol- EO_9 is an alkylphenol analogue of the alcohol ethoxylates, sorbitan trioleate- EO_{20} is similar to the alcohol ethoxylates in being ethoxylated, and A_{12-16} -glucose_{1.6} is similar in containing an alcohol chain of the same average length as the first alcohol ethoxylate $(A_{12-13}-EO_{6.5})$.

The Nomonie Surfactants Scheeted for Testing								
Test Material ²	Brand Name	Supplier	Percent Active ³	Chemical Formula ⁴	Average Molecular Weight ⁵	HLB ⁶		
A ₁₂₋₁₃ EO _{6.5}	NEODOL 23-6.5	Shell Oil	100	CH ₃ (CH ₂) _{11.5} - (OCH ₂ CH ₂) _{6.7} OH	489 (488)	12.1		
A ₁₂₋₁₄ EO ₇	ALFONIC 1412-7	CONDEA Vista	100	CH ₃ (CH ₂) _{12.6} - (OCH ₂ CH ₂) _{7.0} OH	516 (513)	12.0		
A ₈₋₁₀ EO ₅	ALFONIC 810-4.5	CONDEA Vista	100	CH ₃ (CH ₂) _{8.2} - (OCH ₂ CH ₂) _{4.8} OH	358 (356)	12.0		
A ₁₂₋₁₃ EO ₃	NEODOL 23-3	Shell Oil	100	CH ₃ (CH ₂) _{11.5} - (OCH ₂ CH ₂) _{2.9} OH	322 (322)	7.9		
A ₁₂ EO ₂₃ ⁷	BRIJ-35	ICI Specialties	100	CH ₃ (CH ₂) ₁₁ - (OCH ₂ CH ₂) ₂₃ OH	1198	16.9		
Nonylphenol- EO ₀ ⁸	TRITON N101	Union Carbide	100	CH ₃ (CH ₂) ₈ C ₆ H ₄ - (OCH ₂ CH ₂) _{9.0} OH	616 (616)	12.9 ⁵ (13.4)		
Sorbitan tri- oleate-EO ₂₀ 9	TWEEN 85	ICI Specialties	95	$C_{6}H_{9}O_{2}(OCH_{2}-CH_{2})_{20}(C_{18}H_{33}O_{2})_{3}$	1836	11.0		
$A_{12-16} - glucose_{1.6}^{10}$	GLUCOPON 625CS	Henkel	50.2	$\begin{array}{c} CH_{3}(CH_{2})_{11.8}-\\ (C_{6}H_{9}O_{5})_{1.6}OH \end{array}$	455	12.1		
Lauramine oxide ¹¹	INCROMINE OXIDE L	Croda	29.6	CH ₃ (CH ₂) ₁₁ - NO(CH ₃) ₂	229	5.212		
Cocamide DEA ¹¹	STANDAMID KD	Henkel	100	CH ₃ (CH ₂) _{11.8} CO- N(CH ₂ CH ₂ OH) ₂	298	8.812		

Table 10	
The Nonionic Surfactants Selected 1	for Testing ¹

¹ Selected by the Non-animal Testing Research Subcommittee, Soap and Detergent Association.

 2 A = alcohol; the subscript following indicates the carbon chain lengths of the major homologs, i.e., "12-13" = C12 & C13, "12-14" = C12 & C14, and "8-10" = C8 & C10. EO = oxyethylene units; the subscript following indicates the number of oxyethylene units per molecule.

³ Information provided by suppliers (see Appendix 2).

⁴ From CTFA (1977) and information provided by suppliers (see Appendix 2).

⁵Calculated from chemical formula. Molecular weight value, where available from supplier, in parenthesis.

22

⁶ Hydrophilic-lipophilic balance, provided by supplier unless otherwise indicated.

⁷ CTFA name = Laureth-23.

⁹ CTFA name = Polysorbate-85.

- ⁸ CTFA name = Nonoxynol-9. ¹⁰ Alkyl Polyglucoside.

¹¹ CTFA name.

¹² See Figure 5 for method of calculation.

The last two compounds in Table 10 have lower HLB values, like the fourth alcohol ethoxylate tested $(A_{12-13}-EO_3)$ and have alkyl (but not alcohol) chains of similar length. However, they differ in their remaining structural features from the other nonionics tested.

In short, the ten nonionics selected by the Subcommittee represent nonionics typical of those used in the detergent and cleaning products industry selected by structural features and HLB to facilitate structure-activity comparisons.

Figure 5 HLB Calculations Using method of Smith, 1997

HLB = 20 x (mol. wt. hydrophile) / total mol. wt.

Cocamide DEA (mol. wt. = 289)

- Hydrophile = all but alkyl chain (CH3-(CH2)10.8-)
- Hydrophile mol wt. = 298 166 = 132
- HLB = 20 x 132 / 298 = 8.8

Lauramine oxide (mol. wt. = 229)

- Hydrophile = all but alkyl chain (CH3-CH2)11-)
- Hydrophile mol. wt. = 229 169 = 60
- HLB = 20 x 60 / 229 = 5.2

Selection of In Vivo Test Method and Contract Laboratory

One of the purposes of the contract studies was to generate high quality <u>in vivo</u> data on the nonionics selected for testing. Based on the favorable experience of the Subcommittee (Bagley et al., 1994) with an alternative to the standard Draize eye test, the low volume eye test (LVET), this test method was selected as the <u>in vivo</u> test method used in this study. Since Hazleton Wisconsin, Inc., was the contract laboratory successfully used for the <u>in vivo</u> eye irritation testing in previous Subcommittee projects (Neun, 1993; Bagley et al., 1994), Hazleton Wisconsin (now Covance Laboratories) was chosen as the contract laboratory for the LVET.

Since Hazleton Wisconsin had considerable experience in preparing stock solutions for testing, they were also asked to prepare and distribute the stock solutions to the contract laboratories testing surfactant properties. The contact for the Hazleton-Wisconsin study was Mr. Steve Glaza.

Selection of Test Methods and Contract Laboratories for Surfactant Properties

The other purpose of the contract studies was to determine the surfactant properties of the nonionics selected for testing. The following properties were considered:

Surface Tension — Static: A fundamental property of liquids, including water, is their behavior at points of contact (interfaces) with solids, such as a glass surface. The molecules in a liquid have a tendency to adhere to each other rather than to molecules of a solid surface. This resistance to wetting is called the "surface tension" and is operationally defined as "the amount of work required (at a constant temperature) to reversibly increase the surface area of a liquid by a unit amount" (Myers, 1991). In other words, surface tension is the mechanical energy required to cause a liquid to adhere to a solid surface.

Water has a rather high surface tension (73 dynes/cm at 21°C) as can be observed in its tendency to bead up on solid surfaces. As a practical consequence, mechanical energy is required in cleaning to overcome the resistance of water to mixing with and adhering to fabric surfaces.

All surfactants consist of water soluble and oil soluble molecular structures linked together. As a consequence, surfactants have a tendency to accumulate at air-liquid and liquid-solid interfaces, thereby disrupting the self adhesion of the liquid molecules at the interface and reducing the surface tension. Indeed, surfactants are "surface active" because of this tendency to reduce the surface tension of water.

The ability of a surfactant to lower the surface tension of water can be quantified by three parameters: efficiency, effectiveness and critical micelle concentration. The "efficiency" is the concentration of surfactant necessary to reduce the surface tension by 20 units, i.e. to reduce the surface tension of water to 53 dynes/cm. The smaller the amount of surfactant required (on an equal number of molecules or molar basis), the more efficient it is as a surfactant.

The "effectiveness" is the maximum reduction in the surface tension that can be produced by surfactant <u>monomers in solution</u>. The qualifier "monomers in solution" is important because all surfactant molecules have a tendency to self aggregate and form micelles at concentrations above their solubility limits. Further addition of a surfactant above its monomer solubility limit will only form additional/larger micelles, but such micelles are very soluble in water allowing addition of large, or unlimited, amounts of surfactant to the solution without further reducing the surface tension.

The concentration of the surfactant at its monomer solubility limit is called the "critical micelle concentration" (CMC) because that is the concentration at which surfactant micelles begin to form. The CMC is the surfactant concentration at which effectiveness is measured.

Effectiveness and CMC values have already been used to explore structure-activity relationships among in vitro test results on nonionic Surfactants (Part 1, pp. 18-21).

2) <u>Surface Tension — Dynamic:</u> Surface tensions are typically measured at equilibrium or "static" conditions. However, if a new surface is created, for instance by producing a bubble in a water-surfactant solution, a period of time will be required for the surface tension on the bubble surface to reach equilibrium (Myers, 1991).

"Dynamic" surface tension is the surface tension (efficiency, effectiveness and CMC) measured in the time period before equilibrium is reached (generally 0.1 to 10 seconds). Dynamic surface tension can be measured by observing properties of bubbles formed at the rate of 0.1 to 10 bubbles per second (b/s). Increasing the rate of bubble formation decrease the time available for the surfactant to reach equilibrium and thus allows observation of surfactant properties under dynamic conditions. Typically surfactant effectiveness is decreased and CMC increased as the observation period is shortened because the there is less time for equilibrium (optimal) conditions to occur. Conversely, as the observation time increases, surfactant properties approach those measured under equilibrium conditions. Thus both dynamic and equilibrium surfactant properties can be measured in the same experiment by varying the bubble rate.

Surfactant properties during the dynamic (non-equilibrium) time period are determined by static surface tension properties and two additional properties of the surfactant: its diffusion rate and its surface orientation rate (Myers, 1991). The diffusion rate is the speed at which the surfactant molecules move through the water-surfactant solution to reach the new surface. The surface orientation rate is the speed at which the surfactant molecules at the new surface (air-water interface) obtain the most favored orientation, namely with the water soluble portions in the water phase and the oil soluble portions in the air inside the bubble. The diffusion rate is inversely related to the size of the molecule, with larger molecules having slower diffusion rates that smaller molecules. The surface orientation rate is inversely of the molecule, with larger, more complex (e.g. branched) molecules having slower surface orientation rates than smaller, simpler molecules.

3) Surface Tension — Interfacial: Surface tension is typically measured at a water-air interface. However, surface tension can also be measured at a liquid-liquid interface such as that formed by water and a water-immiscible organic liquid. This effect is called the "interfacial surface tension." Surfactants can be expected to reduce the surface tension at the interface depending on their relative solubility in the water and organic liquid phases.

Various organic solvents could be used in an interfacial surface tension measurement: benzene, phenol, mineral oil, or octanol. The latter solvent was chosen for testing since octanol-water partitioning has been used for predicting structure-activity relationships for nonionic surfactants (Part 1 of this report, pp. 22-24; Roberts, 1991). It was hoped that the

interfacial surface tension determined with octanol-water would reveal a structure-activity relationship with this parameter.

4) Surface Tension — Contact Angle: As mentioned above, the ability of a liquid to form a bead on a solid surface is due to surface tension. The angle formed at the interface between the drop of liquid and a flat surface is a measurement of surface tension: the larger (steeper the angle) the higher the surface tension. This angle is called the "contact angle." See Figure 6 for illustration.



A variety of surfaces can be used to measure the contact angle: ceramics/glazed porcelain, glass, plastic laminate, or stainless steel, depending on the hard surface that one wants to model. However, TEFLON tape is now most commonly used because it is chemically inert and has a very smooth and consistent surface (Smith, 1994). Consequently, TEFLON tape was used to measure the contact angle formed by the nonionic surfactants.

The above four parameters (static surface tension, dynamic surface tension, interfacial tension, and contact angle) were considered fundamental measurements of the surfactant properties of nonionic surfactants and were selected for testing the nonionic surfactants list in Table 10. In addition, a number of other surfactant properties of nonionic surfactants were considered:

1) <u>**Cloud Point:**</u> As more and more surfactant is added to a water-surfactant solution (above the CMC), larger/more micelles are formed. With most nonionic surfactants, eventually a

concentration is reached at which the micelles are too large/numerous to stay in solution and a turbid two-phase system (jell) is formed. This phenomenon can also be observed by lowering the temperature of a water-surfactant solution. For solutions containing sufficient concentrations of nonionic surfactants, a temperature will be reached at which the micelles are no longer soluble and the solution will be come cloudy. This temperature is known as the "cloud point."

Cloud point measurements have practical significance for laundry detergents and other concentrated cleaning formulations where a solubilizer (hydrotrope) may be added to prevent the formulation from becoming cloudy at low ambient temperatures (i.e. 40°F) of storage (e.g. Cox, 1990). However, eye irritation testing of surfactants is typically conducted on either 100% active nonionics or on clear aqueous solutions. Consequently, there is no obvious relevance of cloud point to eye irritation potential.

2) Phase Inversion Temperature (PIT): Surfactants, because they have both water-soluble and oil-soluble portions, have the ability to form stable water-and-oil emulsions (Myers, 1991). Stable emulsions can exist in two forms: oil-in-water (o/w) and water-in-oil (w/o). O/W emulsions are formed when the oil is on the inside of the surfactant micelle and the water is on the outside whereas in a w/o emulsion the converse is true. Which type of emulsion is formed depends on the oil phase, the surfactant and the temperature at which the emulsion is held.

Nonionic surfactants are unique in that they will form stable o/w and w/o emulsions of the same oil-and-water combination depending on the temperature of the emulsion. The "phase inversion temperature" (PIT) is defined as the temperature at which an oil-and-water emulsion stabilized with 5% nonionic surfactant will change (invert) from an oil-in-water to a water-in-oil emulsion. The phase inversion temperature is a measure of the stability of the emulsion and is of practical importance in formulating emulsions.

The PIT depends on the oil used to form the system . However, the optimal surfactant for any given oil-and-water system depends on the HLB value of the surfactant (Griffin 1949, 1954). Since the surfactants selected for testing by the Subcommittee were chosen to represent a range of HLB values (Table 10), selection of a single emulsion system which will be optimal for all the nonionics is not possible. Further, measurement of the PIT is technically difficult and requires considerable expertise (Gingell, 1994). For these reasons, the Subcommittee did not attempt to measure PIT on the selected nonionics.

3) **Pour Point / Melting Point / Density:** Many nonionic surfactants, such as alcohol ethoxylates, alkylphenol ethoxylates, ethoxylated sorbitan esters and cocamide DEA, are sold as 100% active materials. A key property for handling these essentially nonaqueous materials is the lowest temperature at which the surfactant can be stored and still remain liquid. This temperature is called the "pour point." This temperature is very similar, if not

identical, to another useful parameter: the minimum temperature at which the solidified surfactant can be melted, the "melting point" (Cox, 1989). Pour point also correlates to the thickness of the surfactant liquid, the "density." Other surfactants, such as alkyl polyglucosides and amine oxides, are sold as aqueous solutions and the pour point/melting point/density is close to that of water (melting point = 0° C, density = 1.0 g/ml).

Consequently, pour point is not useful for predicting eye irritation potential of a group of nonionic surfactants which have different percent actives.

- 4) <u>Viscosity:</u> As some nonionic surfactants, such as alcohol ethoxylates, are diluted with water from 100% active liquids, the surfactant solution forms a jell (mesomorphic) phase at an intermediated dilution which dissolves on addition of more water. This transient jell phase is characterized by high resistance to mixing, i.e. high "viscosity." The concentration range in which the jells are formed and the maximal viscosity in the jell phase are of practical importance in handling such surfactants. However, not all nonionic surfactants display a jell phase on dilution and these nonionics have viscosities similar to water. Consequently, viscosity is not useful for predicting the eye irritation potential of nonionic surfactants other than alcohol ethoxylates.
- 5) **Krafft Point:** The solubility of a surfactant in water depends on the temperature of the surfactant-water solution. As the temperature of the surfactant -water solution is lowered, the solubility of the surfactant is reduced until a temperature is reached at which the surfactant has inadequate solubility to exceed the CMC. The maximum temperature at which surfactant solubility is lower than the CMC is called the "Krafft Point." The Krafft Point is of practical important in formulating anionic surfactants which have limited solubility in water. However, only a few ethoxylated surfactants (nonionics) have solubilities which drop below their CMCs before the freezing point of water is reached. (Schott, 1996). Consequently, only a few nonionics have measurable Krafft Points and this parameter is of little use in predicting the eye irritation potential of nonionics.

<u>Correlations of Surfactant Properties with Structure:</u> Surfactant properties of one class of nonionic surfactants, the alcohol ethoxylates, have been correlated with two structural features: 1) weight percent polyoxyethylene (EO) units (with constant alkyl chain length) and 2) alkyl chain length (with constant weight % EO). The first parameter is related to the hydrophilic-lipophilic balance since HLB is the weight of EO as a percentage of the total nonionic. The second parameter is independent of the HLB. The effects of these two structural parameters on the surfactant properties of alcohol ethoxylates are summarized in Table 11.

Structural Correlations to Surfactante i roper ties of Alconor Ethoxyates						
Surfactant Property ²	Increasing Weight % EO ³ (Constant Alkyl Chain Length)	Increasing Chain Length (Constant Weight % EO ³)				
Surface Tension — Static Critical Micelle Concentration (CMC)	Increases	Decreases				
Surface Tension — Static Efficiency (increases with decreasing CMC)	Decreases	Increases				
Surface Tension — Static Effectiveness (maximal at CMC)	Decreases	Decreases				
Surface Tension — Dynamic CMC	Increases	Increases				
Surface Tension — Dynamic Effectiveness (maximal at CMC)	Decreases	Decreases				
Cloud Point	Increases	Increases (due to lower free alcohol), then decreases				
Phase Inversion Temperature	Increases	No effect				
Pour Point/Melting Point/Density	Increases	Increases				
Viscosity	Decreases (due to increasing water solubility), then increases	Increases				

Table 11 Structural Correlations to Surfactant Properties of Alcohol Ethoxylates¹

¹ From Cox (1989) and Myers (1991).

² Structural properties are defined and described in the text.

 3 EO = oxyethylene units.

The correlations shown in Table 11 can be explained based on physical-chemical properties of the surfactants (Cox, 1989; Myers, 1991, Smith, 1994). For instance, increasing the chain length (constant HLB) increases the molecular weight and decreases the water solubility of the molecule, decreasing the CMC. As CMC decreases, surfactant efficiency increases because it takes less surfactant to reach the CMC. Surfactant effectiveness decreases since fewer surfactant molecules can pack into the same interfacial area. Dynamic CMC increases (and effectiveness decreases) because it takes longer for larger molecules to reach equilibrium. Pour point, melting point and density increase due to increased intermolecular interactions (Van der Waals forces), especially between the ethoxylate side chains. The viscosity increases since it is more difficult for larger molecules to slip past each other.

In contrast, increasing the weight % EO (constant alkyl chain length) increases the water solubility, increasing the cloud point, phase inversion temperature and CMC. As CMC increases, surfactant efficiency decreases because it takes more surfactant to reach the CMC.

Information available from the suppliers (collected in Appendix 2) on the surfactant properties of the nonionics selected for testing by the Subcommittee are summarized in Tables 12 and 13. In addition to the static surface tension data listed in Table 12, data on interfacial surface tension (versus mineral oil) is available from Henkel on GLUCOPON 625CS (A_{12-16} -glucose_{1.6}). As one can see, there is no data available on dynamic surface tension or contact angle. The data on static surface tension is limited to only three of the 10 surfactants selected and some of this data is limited to approximate values.

			V
Nonionic Surfactant ²	Surface Tension — Effectiveness ³	Surface Tension — CMC⁴	Surface Tension — Efficiency⁵
A ₁₂₋₁₃ EO _{6.5} ⁶	28	17 ppm	1.1 ppm
	dynes/cm	(35 μM)	(0.15 μM)
Nonylphenol-	30	~100 ppm ⁹	<5.6 ppm
EO ₉ 7	dynes/cm	(160 µM)	(<9.1 μM)
$A_{12-16} - glucose_{1.6}^{8}$	29.3	30 ppm	<100 ppm
	dynes/cm	(66 μM)	(<12 μM)

Table 12Surface Tension Properties of Nonionics Reported by Suppliers1

¹ See Appendix 2 for supplier information. See text for explanation of properties.

² See Table 10 for full description of nonionics.

³ Surface tension at critical micelle concentration (CMC).

⁴ parts per million (ppm) active (weight/weight basis) or micromoles per liter (μM).

⁵ Concentration (in ppm or μ M) for decrease in surface tension by 20 dynes/^{cm}. Calculated by simple proportion from surface tension reduction at most dilute concentration tested.

⁶ At 76°F (24.4°C) in distilled water. ⁷ At 25°C in water.

⁸ At 25°C in deionized water.

⁹ Estimated from surface tension versus concentration data.

As shown in Table 13, the situation is somewhat better for the other surfactant properties considered by the Subcommittee. The data set is complete for four of the five alcohol ethoxylates and there is pour point of density data on all the nonionics. These is also viscosity data on seven of the ten surfactants, and cloud point data on six of the ten. However, the viscosity data were collected at two different temperatures complicating comparisons, and the range of reported densities is rather narrow (0.92-1.05). No phase inversion temperature data was found for any of the surfactants.

						A
Nonionic Surfactant ²	Cloud Point ³	Pour Point	Melting Point/ Range	Density ⁴	Viscosity ⁵	Solubility ⁶
A ₁₂₋₁₃ EO _{6.5}	113°F (45°C)	59°F (15°C)	39-70°F (4-21°C)	0.984 @77°F (25°C)	29 cP @100°F (38°C)	
A ₁₂₋₁₄ EO ₇	53.9°C	66°F (19°C)	66-77°F (19-25°C)	0.980 @22°C	35 cP @38°C	
A ₈₋₁₀ EO ₅	37.5°C	34°F (2°C)	32-35°F (0-2°C)	0.983 @22°C	16 cP @38°C	
A ₁₂₋₁₃ EO ₃	33.1°F (0.5°C)	34°F (2°C)	19-37°F (-7-3°C)	0.922 @77°F (25°C)	14 cP @100°C (38°C)	"partial insoluble at 1%"
A ₁₂ EO ₂₃		91°F (33°C)		1.05 g/cc @25°C		"soluble"
Nonylphenol- EO ₉	54°C	40°F (4°C)		1.046 @25°C	240 cP @25°C	"soluble in all portions"
Sorbitan tri- oleate-EO ₂₀		-20°F (-29°C)		~1.0 @25°C	~300 cP @25°C	"dispersible"
$\begin{array}{c} A_{12-16} - \\ glucose_{1.6} \end{array}$	>100°C	12°C			21500 cP @ 25°C 6250 cP @ 35°C	"dispersible"
Lauramine oxide				0.96 vs. water = 1.0		"soluble"
Cocamide DEA		20°C				"insoluble but dispersible"

Table 13Additional Properties of Nonionic Surfactants Reported by Suppliers1

¹ See Appendix 2 for supplier information. See text for explanation of surfactant properties. Blanks in table indicated no data.

² See Table 10 for full description of nonionics. ³ Of 1% solution in water.

⁴ Density (in g/cc) at the indicated temperature or specific gravity (dimensionless) as the ratio of the density of the

nonionic at the indicated temperature versus that of water at the same temperature.

⁵ In centipoise (cP = cSt).

⁶ in water

The small amount of data available confirms the wisdom of the Subcommittee's decision to generate the key surface tension data on all of the nonionic surfactants selected for testing.

<u>Contract Laboratories</u>: A search was made by Subcommittee members to identify contract laboratories to generate the surface tension data. CONDEA Vista (Austin, Texas, formerly Vista Chemical Company, contact: Mr. Dewey Smith) volunteered to conduct surface tension

(equilibrium) measurements on solutions of the nonionics. CONDEA Vista has published a number of studies on surfactant properties (e.g. Cox, 1989, 1990) and are technical experts at such measurements. Consequently, their offer was accepted by the Subcommittee.

The United States Testing Company, Inc., of Hoboken, New Jersey (Chemical Services Division, Contact: Mr. Joseph Kwiatkowski, Assistant Vice President) was identified as a contract laboratory which could determine interfacial tension and contact angle as well as static surface tension. This laboratory has an excellent reputation and was accepted by the Subcommittee for testing of surface tension, interfacial tension and contact angle.

Only one contract laboratory was identified for dynamic surface tension measurements: SensaDyne Instrument Division of the Chem-Dyne Research Corporation, Mesa, Arizona (contact: Mr. Victor Janule, Division President). SynsaDyne developed and sells instruments to measure dynamic surface tension by measuring the properties of bubbles formed at various rates in water-surfactant solutions (see above discussion on Surface Tension -- Dynamic). This instrument can also estimate static surface tension by using slow rates of bubble formation. However, such values may not be identical to surface tension measurements determined with standard instrumentation. Since SynsaDyne conducts contract studies, they were accepted by the Subcommittee for testing dynamic and static surface tension, including effectiveness, CMC, and efficiency measurements.

Selection of Nonionic Surfactant Doses for Testing

As with the previous testing conducted by the Non-animal Testing Subcommittee (Bagley et al., 1994), the nonionics surfactants selected for testing were to be evaluated in aqueous solutions at concentrations representative of those in detergent and cleaning products. This would avoid the difficulty of attempting to correlate the results of <u>in vivo</u> eye irritation results on essentially 100% active (nonaqueous) materials to <u>in vitro</u> eye irritation results derived from testing aqueous solutions. Moreover, it would avoid the extreme difficulty of attempting to measure surfactant properties of nonaqueous solutions.

It was the judgement of the Subcommittee that 10% aqueous solutions were about the maximum concentration that would be realistic. This is the typical concentration of detergent and cleaning product ingredients tested by Bagley et al. (1994). Based on the molecular weights in Table 10, such solutions would range in molarity from 0.08 M ($A_{12}EO_{23}$) to 0.4 M (lauramine oxide).

It also would simplify the analysis if all the nonionic surfactants were tested at an equimolar concentration. This would allow direct comparison between the results obtained with the materials at the same molecular concentration.

Indeed, it would also simplify the analysis if the same surfactant concentrations were tested in the in vivo eye irritation test (low volume eye test, LVET) as in the surfactant properties (surface

tension) tests. This would allow a direct comparison of eye irritation results with surfactant property results.

With that agreed, the decision facing the Subcommittee was to determine the optimal dose to test. On one hand, the solubility of some of the surfactants in water is somewhat limited although precise data on the limits of solubility are not available. (See Appendix 2.) On the other hand, surfactant solutions had to be of sufficient strength to produce at least some irritation in the LVET and to allow some judgement to be made regarding the surfactants with the most and least eye irritation potential.

Information from the suppliers on the solubilities of the nonionic surfactants are shown in Table 13. It is clear from an examination of the data that it is not possible to select an aqueous concentration low enough to allow all the nonionics to be soluble. For instance, the low-mole alcohol ethoxylate A_{12-13} -EO₃ is not completely soluble even at a 1% solution. Cocamide DEA is described as "insoluble" but can be dispersed in water, as is true of several other of the nonionics.

As previously shown in Table 3, the eye irritation potential of some nonionic surfactants is greatly reduced by dilution. Consequently, it was decided to test 0.2 \underline{M} solutions since these would be approximately equal to a 10% solution of the first two alcohol ethoxylates in Table 10.

The expected maximum average score (MAS) in the standard Draize eye irritation test of the 0.2 <u>M</u> solutions are shown in Table 14. Predicted MASs of the nonionic surfactants range from very low scores (<4) to moderately severe scores (59-69).

Based on the results in Bagley et al. (1994), the <u>in vivo</u> test to be used with these surfactants (low volume eye test, LVET) can be expected to give somewhat lower scores. Based on the regression curve between maximum average scores in the Draize test (DMAS) and in the LVET (LMAS) shown in Figure 1 from Bagley et al. (1994), the relationship for irritants having MASs in the range of 0 to 40 is: LMAS = 0.86 (DMAS) - 6. Consequently, MAS results in the LVET are expected to range from 0 to about 53. This wide range of MAS scores is expected to give good separation between less irritating and more irritating nonionics.

METHODS

Preparation of Test Solutions

Samples of each of the nonionic surfactants listed in Table 10 were provided to the Hazleton-Wisconsin testing laboratory. Each sample consisted of at least 100 grams of material. Samples were provided by: Ralph Gingell (NEODOL alcohol ethoxylates), John Heinze (ALFONIC alcohol ethoxylates) and Marie Capdevielle (all others).

Fleuicieu Maximum Average Draize Scores for Nomonic Surfactants						
Test Material ²	Predicted MAS ³ at 0.2 <u>M</u>	Based on: (Tested Compound)	Molecular Weight⁴	Tested Concentration ⁴	Reported MAS ^{3,4}	Ref. ⁵
A ₁₂₋₁₃ EO ₆₅	10-35	A ₁₃ EO ₆	465	10% (0.22 <u>M</u>)	10-35	1
A ₁₂₋₁₄ EO ₇	15	A ₁₂₋₁₄ EO ₇	516	10% (0.19 <u>M</u>)	14.7	2
A ₈₋₁₀ EO ₅	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶
A ₁₂₋₁₃ EO ₃	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶	N.A. ⁶
$A_{12}EO_{23}^{7}$	<12	A ₁₂₋₁₄ EO ₂₃	1209	Undiluted (0.83 <u>M</u>)	12.3	3
Nonylphenol- EO ₉ ⁸	59-69	Octylphenol- EO ₉ ⁷	602	10% (0.17 <u>M</u>)	59, 69	4, 5
Sorbitan tri- oleate-EO ₂₀	1-4	Sorbitan laurate-EO ₂₀ ⁸	1192	Undiluted (0.84 <u>M)</u> 10% (0.084 <u>M</u>)	4 1	4
$A_{12-16} - glucose_{1.6}$	<4	$A_{12-16} - glucose_{1.6}^{9}$	456	15% (0.33 <u>M</u>)	4	6
Lauramine oxide	15-"severe"	Lauramine oxide	229	100% (4.4 <u>M</u>) ¹⁰ 1% (0.044 <u>M</u>)	"severe" 14.8	72
Cocamide DEA	<22-23	Lauramine DEA	287	10% (0.35 <u>M</u>)	22, 23	8

Table 14Predicted Maximum Average Draize Scores for Nonionic Surfactants1

¹ Nonionic surfactants selected for testing by the Non-animal Testing Research Subcommittee, Soap and Detergent Association.

² A = alcohol; the subscript following indicates the carbon chain lengths of the major homologs, i.e., "1213" = C12 & C13, "1214" = C12 & C14, and "810" = C8 & C10. EO = oxyethylene units; the subscript following indicates the number of oxyethylene units per molecule.

 3 MAS = maximum average eye irritation score in the standard Draize test (on scale of 0 to 110).

⁴ of compound tested.

⁵ Ref. = reference:	
1 = Benke et al., 1977.	2 = Bagley et al., 1994.
3 = Procter & Gamble, unpublished: See D. J.	Innis in Appendix 1.
4 = ECETOC, 1992.	5 = Kennah et al., 1989.
6 = Henkel Corp., unpublished.	7 = NIOSH, 1993.
8 = Cosmetic Ingredient Review, 1986.	
⁶ N.A. = not available.	⁷ TRITON X100.
⁸ TWEEN 20.	⁹ APG 550.
¹⁰ "50 μ g" (powder?) tested.	

Preparation of test materials is described in detail in the Final Report on the study by Mr. Steve Glaza of Hazleton-Wisconsin (Appendix 3).

Each test material was prepared as a 0.2 <u>M</u> solution/suspension by diluting the appropriate amount of nonionic surfactant with deionized water. The pH of the test solutions were determined (see Appendix 4 for raw data). While most of the test solutions were neutral to slightly acidic (pH 3.1 to 7.4), two of the test solutions (A_{12-16} -glucose_{1.6} and cocamide DEA) were somewhat alkaline (pH 9 to 10).

Previous testing conducted by the Subcommittee (Neun, 1993) demonstrated that the eye irritation potential of an aqueous solution is determined by its pH and alkalinity. To determine if the eye irritation potentials (and surfactant properties) of nonionic surfactants are influenced by slightly alkaline pHs, lauramine oxide solutions were prepared and adjusted to two pH values, 7.0 and 10.5.

Test materials were prepared fresh on the day of dosing (for <u>in vivo</u> testing) and stored at room temperature until administered in the LVET. A 110-mL subsample of each test material, stored at room temperature, was shipped two days after preparation to the contact persons at the surfactant property testing laboratories selected by the Subcommittee (see Contract Laboratories).

Low Volume Eye Test (LVET) Procedures

Test procedures are described in detail in the Final Report issued by Steve Glaza of Hazleton-Wisconsin (Appendix 3).

Each 0.2 <u>M</u> test mixture was administered to a group of three health adult albino rabbits. Each rabbit received 10 μ L of the test material mixture placed directly on the corneal surface of the right eye, with the left eye serving as the untreated control. The eyelids were released without holding them shut or forcing blinking. The eyes were not flushed after treatment.

The treated eyes of all animals were observed for ocular irritation at 1, 24, 48, 72 and 96 hours after treatment. Scoring was discontinued for each group once all treated eyes within that group cleared of irritation. After recording the 24-hour observations, sodium fluorescein was used to aid in revealing any possible corneal injury. Irritation was graded according to the Draize technique using a penlight as the source of illumination. Any eye abnormalities were recorded.

Surfactant Property Measurement Procedures

CONDEA Vista Company: Equilibrium surface tension measurements were conducted on test solutions at 25°C using an automatic Lauda tensiometer. This instrument is basically an automated version of the manual du Nuoy ring method. In this procedure, a thin platinum wire (du Nuoy ring) attached to a pull rod is lowered below the surface of the test solution. The ring is automatically pulled through the air-solution interface until the maximum pull on the ring is measured. The surface

tension is calculated from the maximum force measured, the geometry of the ring and the density difference between air and the test solution. See Appendix 5 for a more detailed description of the procedure used.

Because of high viscosity of three test materials (sorbitan trioleate- EO_{20} , A_{12-16} -glucose_{1.6}, and cocamide DEA), these materials were diluted 1 to 3 with deionized water prior to surface tension measurement. Dilution was accomplished by adding 15 mL of test material to 45 mL of deionized water. CONDEA Vista reported that this dilution gave a solution sufficiently fluid to measure surface tension in their apparatus.

Dilution of the test material is valid only if the dilution does not change the surface tension, i.e. the surfactant concentration remains above the CMC. To test the validity of the dilution, a further 5 mL of deionized water was added to each of the three test solutions to give a 3 to 10 dilution, and the surface tension re-measured.

<u>United States Testing Company:</u> Surface tension and interfacial tension were determined according to the procedures in ASTM D-1331 (Appendix 6) using a du Nuoy tensiometer. This instrument is basically a torsion balance with the arm connected to the pull rod of the du Nuoy ring and a dial to adjust the force on the arm. The dial is calibrated by adjusting the length of the torsion arm so that the scale on the dial reads the force in dynes/cm. The ring is lowered below the test solution-air interface (surface tension) or test solution-octanol interface (interfacial tension). The ring is slowly raised by turning the calibrated dial until the ring breaks free of the interface. Surface tension is calculated from the maximum force measured, the geometry of the ring and the density difference between air and the test solution. Interfacial tension is calculated from the maximum force measured, the geometry of the ring and the density difference between the test solution and octanol. A correction factor is applied to both measurements to account for the nonplanar contour of the test solution surface touching the du Nuoy ring at the instant of breakaway. The correction factor is calculated from the geometry of the wire and the density difference between the test solution and air (surface tension) or octanol (interfacial tension).

Contact angle measurements were made with a drop projection instrument (Kayeness Model D1060) following the procedure in ASTM D-724 (Appendix 6). TEFLON tape was used as the substrate. Basically, this instrument projects the image of a drop (approximately 5-7 μ L) of the test solution on the substrate surface onto a sheet of paper, where the tangent line (see Figure 6) can be drawn and measured with a protractor.

<u>SensaDyne Instrument Division</u>: Dynamic surface tension was measured using the PC9000 surface tensiometer (See Appendix 7). Like all SensaDyne tensiometers, this instrument measures the difference in gas pressure between two capillary tubes having different orifice sizes. The capillary tubes and a temperature probe are immersed in the test solution at the same depth. Independent flow controllers are used to force nitrogen gas through both orifices at the same rate of bubble formation. The bubble rate may be varied from one bubble every 30+ seconds to 40+ bubbles per second.
The pressure, P, necessary to form a bubble in solution is given by the equation: $P - P_0 = 2S/r + dhg$, where P_0 is the atmospheric pressure, S is the surface tension, r is the radius of the orifice, d is the solution density, h is the capillary height (depth), and g is the gravitational constant (See papers by S. M. Hosseini and V. P. Janule in Appendix 7). When comparing the pressure difference between the two orifices, all terms but P, S and r cancel out so that the pressure difference is proportional to the surface tension: $S = (P_1r_1 - P_2r_2)/2$.

The procedure for determining dynamic surface tension is described in detail in the report from SensaDyne Instrument Division ("Physical & Chemical Properties of Nonionic Surfactants: Surface Tension Study - HWI#6310-105, Appendix 7).

Dilute surfactant solutions were prepared by carefully filling a graduated beaker to the 100-mL mark with deionized water and adding 400 μ L of test material with a 500- μ L graduated syringe. After mixing for a minimum of 30 seconds, probes from the SensaDyne PC9000 surface tensiometer were placed in the surfactant solution and the surface tension reading recorded after the values had stabilized. The sample was then removed and the probes cleaned. After the sample was re-mixed, a second reading was recorded. Duplicate readings were obtained after the addition of each 400 μ L portion of test material until surface tension readings no longer changed and no further lowering of surface tension was evident.

Each of the test materials was tested at four bubble rates to generate a series of surface tension curves. Prior to each of the four runs the instrument was set at a predetermined bubble rate (in bubbles per second, b/s) in water and calibrated using deionized water and ethanol (Spectrum Chemical Corp., ET107). The calibration was then rechecked for accuracy prior to each new sample being tested at that rate.

TEST RESULTS

Preparation of Test Solutions

A comparison of the actual molecular weights of the nonionic surfactants (from Table 10) with the values supplied by the Subcommittee and used by Hazleton-Wisconsin reveals some discrepancies As shown in Table 15, these discrepancies resulted in some of the test solutions being prepared at molar concentrations different from the target value of 0.2 M. In most cases, the differences are only a few percent and can be ignored. However, in the case of sorbitan trioleate-EO₂₀, the test solution concentration (0.06 M) is only one-third of the target value, and in the case of $A_{12}EO_{23}$, the test solution concentration (0.14 M) was two-thirds of the target value. The impact of these differences on other test results will be discussed below.

Hazleton-Wisconsin reported (Appendix 3, page 6) that all test material solutions/suspensions appeared to be homogenous when prepared and used. United States Testing Company noted

(Appendix 6, report dated 2/1/95, page 2) the presence of "small solid matter" in sample #8 (A₁₂₋₁₆ - glucose_{1.6}). This material seems to be debris from preparation of the test solution and would not be expected to interfere with surface tension measurements. The company did not note any interference in measurements with this test solution.

Nonionic Surfactant ²	Actual Molecular Weight ³	Molecular Weight Used⁴	Actual Molarity of Test Solutions ⁵
A ₁₂₋₁₃ EO _{6.5}	488	463	0.190 <u>M</u>
A ₁₂₋₁₄ EO ₇	513	512	0.200 <u>M</u>
A ₈₋₁₀ EO ₅	356	366	0.206 <u>M</u>
A ₁₂₋₁₃ EO ₃	322	317	0.197 <u>M</u>
$A_{12}EO_{23}$	1198	846	0.141 <u>M</u>
Nonylphenol- EO9.	616	616	0.200 <u>M</u>
Sorbitan tri- oleate-EO ₂₀	1836	556	0.061 <u>M</u>
$A_{12-16} - glucose_{1.6}$	455	453	0.199 <u>M</u>
Lauramine oxide	229	229	0.200 <u>M</u>
Cocamide DEA	298	305	0.205 <u>M</u>

Table 15
Actual Test Solution Concentrations of Nonionic Surfactants ¹

¹ Actual molarity of surfactant solutions (target = 0.2 M) based on molecular weights in Table 10 and procedures used by Hazleton-Wisconsin. Molecular weights used by Hazleton-Wisconsin were supplied by the Subcommittee. ² See Table 10 for full description of nonionics.

³ From Table 10. Molecular weight values provided by suppliers used if different from calculated values.

⁴ From table of Test Material Preparation Calculations in Appendix 4.

⁵ Calculated by dividing the grams of test material used (Test Material Preparation Calculations in Appendix 4) by 0.4 L (volume of solution prepared) and actual molecular weight.

Results from the Low Volume Eye Test (LVET)

Descriptive Analysis: Low volume eye test results on nonionic surfactants are described in detail in the Final Report from Steve Glaza at Hazleton-Wisconsin (Appendix 3) while the raw data is included in Appendix 4. Table 16 (below) summarizes the results.

Summary Results from Low Volume Eye Test on Nomonic Surfactants					
Nonionic Surfactant ²	Test Concentration ³	Maximum Individual Score ⁴	Maximum Average Score ⁵	Days To Clear ⁶	Median Days To Clear ⁷
A ₁₂₋₁₃ EO _{6.5}	0.19 <u>M</u>	4, 6, 11	7.0	3, 4, 4	4
A ₁₂₋₁₄ EO ₇	0.20 <u>M</u>	2, 4, 8	4.7	3, 3, 3	3
A ₈₋₁₀ EO ₅	0.21 <u>M</u>	8, 9, 17	11.3	2, 2, 4	2
A ₁₂₋₁₃ EO ₃	0.20 <u>M</u>	0, 0, 0	0.0	0.04, 0.04, 0.04	0.04
A ₁₂ EO ₂₃	0.14 <u>M</u>	0, 2, 2	1.3	0.04, 1, 1	1
Nonylphenol- EO ₉	0.20 <u>M</u>	8, 11, 21	13.3	4, 4, 4	4
Sorbitan tri- oleate-EO ₂₀	0.06 <u>M</u>	0, 0, 2	0.7	0.04, 0.04, 1	0.04
$\begin{array}{c} \mathbf{A}_{12-16} -\\ \mathbf{glucose}_{1.6} \end{array}$	0.20 <u>M</u>	2, 11, 13	8.7	1, 1, 1	1
Lauramine oxide (pH=7.0)	0.20 <u>M</u>	9, 13, 13	11.7	2, 2, 3	2
Lauramine oxide (pH=10.5)	0.20 <u>M</u>	13, 13, 13	13.0	1, 1, 1	1
Cocamide DEA	0.20 <u>M</u>	2, 9, 11	7.3	1, 1, 1	1

Table 16Summary Results from Low Volume Eye Test on Nonionic Surfactants1

¹ Results are taken from Final Report of Steven M. Glaza, Hazleton Wisconsin, Inc. (Appendix 3). Data from Table 2 of Final Report unless otherwise indicated.

² See Table 10 for full description of nonionics. ³ From Table 15.

⁴ Individual animal primary eye irritation scores, in ascending order, from observation period (1 hour) giving the maximum average score. Data from Appendix of Final Report.

⁵ The highest average eye irritation score recorded for any given observation period.

⁶ Time in days, in ascending order, for individual animals to clear of all eye irritation. Note that 1 hour = 0.04 days.

⁷ The middle value from the individual clearance times.

Maximum average scores (MAS) in the low volume eye test range from 0 to 13.3 on an irritation scale that ranges from 0 (no eye irritation) to 110 (maximum possible score). Maximal individual animal scores were all observed one hour after treatment, with no higher scores at 24 hours or any later time of observation. The median number of days to clear, for animals with measurable eye irritation, ranged from one hour to four days. All eye irritation had cleared by 4 days of treatment. Considering the low MAS values observed and the transient nature of the irritation observed, the eye

irritation potential of the surfactants solutions evaluated in this test ranged from none to low (MAS<15). Since the surfactant concentrations in the test solutions (0.20 <u>M</u> target) was chosen to approximate realistic maximal concentrations in detergents and cleaning products (see section on "Selection of Nonionic Surfactant Doses for Testing" above), the eye irritation potential of nonionic surfactants tested at use concentrations in detergents and cleaning products is low.

Based on the eye irritation results for structurally similar surfactants in the standard Draize eye test (Table 14) and the relationship between the LVET and the standard Draize test observed in Bagley et al (1994), MAS scores in the LVET were expected to range from 0 to about 53. As shown in Table 16, MAS scores with the nonionics actually ranged from 0 to 13.3, a much narrower range of values. Thus quantitatively, these values are lower than expected.

However, the test results reported in Table 16 do not appear to be unreasonable compared to previously reported irritation scores in the low volume eye test. For instance, 14 of the 22 test materials studied by Bagley et al. (1994) gave maximum average scores in the LVET of 0 to 13.7, the range of MAS values reported in Table 16. As a more direct comparison, Bagley et al. (1994) reported a maximum average score of 8.7 and a median days to clear of 3 for $A_{12-14}EO_7$ (10% = 0.19 M) in the low volume eye test. These results are similar to those reported in Table 16 for $A_{12-14}EO_7$, where MAS = 4.7 and MDTC = 3. It should be noted that these two tests were conducted at the same laboratory, Hazleton Wisconsin, using nominally identical surfactants. (The exact description of the material tested by Bagley et al. has been lost.)

Hazleton Wisconsin also conducted a repeat test on $A_{12-14}EO_7$ (0.20 <u>M</u>) in the standard Draize eye test because of the questions about the exact identity of the material tested by Bagley et al. (1994). The results, included in the report from Steve Glaza in Appendix 3, are MAS = 17.3 and MDTC = 8.5. These results are similar to those reported by Bagley et al. (1994) for $A_{12-14}EO_7$ of MAS = 14.7 and MDTC = 4.

Quantitatively, the eye irritation scores in Table 16 are somewhat lower than expected but reasonably consistent with previous results on a nominally similar material tested in the low volume eye test at the same laboratory.

Qualitatively, however, the results in Table 16 are quite consistent with the expected results predicted in Table 14. For instance, the two nonionics predicted in Table 14 to be give the highest MAS scores (nonylphenol-EO₉ and lauramine oxide) have the highest MAS scores in Table 16 (13.3 and 13.0, respectively). Two nonionics predicted to give low MAS scores in Table 14 ($A_{12}EO_{23}$ and sorbitan trioleate-EO₉) have low MAS scores in Table 16 (1.3 and 0.7, respectively). The three nonionics predicted to give intermediate scores in Table 14 ($A_{12-13}EO_{6.5}$, $A_{12-14}EO_7$ and cocamide DEA) gave intermediate MAS scores in Table 16 (7.0, 4.7 and 7.3, respectively).

The one exception to the predicted pattern is instructive. A_{12-16} -glucose_{1.6} is predicted to give a MAS score <4 based on unpublished results from testing of a similar material at a somewhat higher

concentration. However, the Material Safety Data Sheet on the material tested in the LVET (GLUCOPON 625CS) reports that when the undiluted (50% active, 1.1 M) material was tested in the standard Draize eye irritation test, maximum individual scores ranged from 39 to 61 (see Appendix 2). Apparently, there are differences in the eye irritation potential of APG 550 (Table 14) and GLUCOPON 625CS that would not be predicted by the close similarities of the structures of these materials.

It should be noted that two of the three nonionics which gave the lowest maximum average scores $(A_{12}EO_{23} \text{ and sorbitan trioleate-}EO_9)$ were the two nonionics which were inadvertently tested at lower molar concentrations than the other nonionics. This may have contributed to the low MAS results obtained with these materials. However, the results cited in Table 14 for closely related materials suggest than low MAS results would have been observed for these materials even if higher concentrations had been tested.

Statistical Analysis: As shown in Table 17, there is a highly significant difference (p < 0.001) in the maximum average scores for the surfactants tested. These differences were determined using analysis of variance (ANOVA), and a full print out of the analysis is included in Appendix 8.

tati	stical Analy	sis: Maximu	im Individ	ual Draize	Scores	of Test Solut	ion
	Source of Variation	Degrees of Freedom ²	Sum of Squares ³	Mean Squares⁴	F ⁵	Significance Level ⁶	
	Surfactants	10	737.5758	73.7576	5.1459	0.0007	

14.3333

 Table 17

 Statistical Analysis: Maximum Individual Draize Scores of Test Solutions¹

¹ Analysis of variance of nonionic surfactants (test solutions) and the maximum individual scores in the low volume eye test (See Table 16 for data). Statistical analysis using the STAT PACKETS computer program (One Factor Completely Randomized Design). The full print out of the data set and report is included in Appendix 8.

315.3333

1052.9091

² Degrees of freedom (DF) = N - 1, where N = 11 test solutions (surfactants) or 33 individual scores (total). DF for error = DF(Total) - DF(Surfactants).

³ The sum of squares is a measure of the variance. The variance due to error is the total variance less the surfactants variance.

⁴ Mean squares are the sum of squares divided by the DF.

22

32

Error

Total

⁵ F is the ratio of the mean squares for surfactants divided by the mean squares for error.

⁶ The significance level or probability value is calculated from F and DF.

Based on the ANOVA results, significant differences between the maximum average scores between pairs of surfactants were determined using a t-test in which no assumption was made as to which surfactant would give the higher MAS value, i.e. a "two tailed" test was used. Detailed results are

given in Appendix 8 and summarized in Table 18, where the surfactants are listed in order of increasing values of MAS. Significant differences between surfactants are indicated by the letters following the MAS values; MAS values sharing the same letter are not significantly different.

Nonionio Surfactant ²	MAS ³	Significant Differences ⁴	Group ⁵
	0.0	2	T.
A ₁₂₋₁₃ EO ₃	0.0	a	
Sorbitan trioleate-EO ₂₀	0.7	ab	L
A ₁₂ EO ₂₃	1.3	abc	L
A _{12,14} EO ₇	4.7	abcd	L
A _{12,12} EO _{6,5}	7.0	bcde	I
Cocamide DEA	7.3	cde	I
A ₁₂₋₁₆ - glucose _{1.6}	8.7	de	I
A _{e 10} EO ₅	11.3	e	Н
Lauramine oxide (pH=7.0)	11.7	e	Н
Lauramine oxide (pH=10.5)	13.0	e	Н
Nonviphenol-EO ₀	13.3	е	Н

 Table 18

 Significant Differences among Low Volume Eye Test Scores of Surfactants¹

¹ Statistical differences between maximum average scores of nonionic surfactants as determined by analysis of variance. See Table 17 and Appendix 8 for details.

² See Table 10 for full description of nonionics. ³ Maximum average scores (MAS) from Table 16.

⁴ MAS values with the same letter are not significantly different at the p = 0.05 probability level (T-Test). ⁵ Surfactants are grouped by their relative MAS results, where "L" is the lowest group, "I" is an intermediate group and "H" is the highest group. Note that the surfactants in group L and H have significantly different MAS values while the group I values are not significantly different from the low and high groups.

This is a rather complicated pattern of statistical differences. However, the analysis can be simplified by observing that the four nonionics giving the lowest MAS values ($A_{12-13}EO_3$, sorbitan trioleate-EO₉, $A_{12}EO_{23}$ and $A_{12-14}EO_7$) comprise a group in which the MAS results are significantly lower than those of the group of four nonionics which give the highest MAS results ($A_{8-10}EO_5$, lauramine oxide (pH=7.0), lauramine oxide (pH=10.5) and nonylphenol-EO₉). The remaining three nonionics ($A_{12-13}EO_{6.5}$, cocamide DEA, and A_{12-16} -glucose_{1.6}) comprise a group with intermediate MAS values not significantly different from the surfactants in the low and high MAS groups.

It should be clear that "low," "intermediate" and "high" in this context are terms relative to this method of grouping the MAS results. As mentioned previously, the eye irritation potential of all the surfactants tested appears to be low (see Table 16).

It should be noted that the results from this statistical analysis are somewhat different from those previously reported to the Subcommittee (J. Heinze, memo to J. Al-Atrash, March 16, 1995, see Appendix 8). The latter analysis used confidence intervals calculated from the standard deviation of the MAS values. However, the value (1.23) used to calculate the 95% confidence intervals from the standard deviation was taken from an earlier analysis of the Subcommittee's Phase III results [Booman, 1994, Appendix 8], and is incorrect since this value is based on the standard Draize eye test method with 6 animals per test material instead of the 3 animals per test material used in the low volume eye test.

The statistical analysis used in this report (ANOVA followed by paired two-tailed t-tests) is a more conservative and more widely accepted approach to the analysis of the MAS scores than the approached previously attempted. Fortunately, the current method of analysis actually gives a better separation of the nonionics into the three groups (low, intermediate and high) than the previous (and incorrectly applied) approach. Consequently, no attempt has been made to re-analyze the data using the previous approach.

As shown in Table 16, median days to clear gives a smaller range of values (0.04 to 4 days) than does the MAS scores (0 to 13.3). Moreover, MDTC is a discrete, rather than a continuous scale because reading are made only at pre-determined times, i.e., 1 hour, 24 hours, 48 hours, etc. Consequently, analysis of significant differences among MDTC scores requires use of non-parametric methods of statistical analysis beyond the scope of this report.

Figure 7 shows a parametric analysis of the correlation between the maximum average score (MAS) and the median days to clear. A linear regression line can be drawn but the correlation coefficient is low ($R^2 = 0.25$). The regression line is in fact not significant (p>0.05) when analyzed by the t-test as described under "Structure-Activity Relationships Among Alcohol Ethoxylates." No further analysis of the MDTC scores was attempted.

<u>Correlations to Physical Properties / Structure</u>: Physical properties, such as the pH's of the test solutions and the hydrophilic-lipophilic balances of the nonionic surfactants, as well as the structural features of the nonionics, can now be examined for their ability to predict the observed eye irritation potential of the test solutions. The data to be used for these comparisons are summarized in Table 19.

Possible correlations between the pH's of the test solutions and the eye irritation results were first examined by Fred Heitfeld and his analysis is included in Appendix 8 (attached to memo from J. Al-Atrash, October 25, 1995). In this analysis, Mr. Heitfeld plotted both the maximum average score

("Max Av. Drz") and the median days to clear ("DTC") versus the pH of the test solution (Figure titled "Effect of pH on Eye Irritation"). He concluded that there was no clear relationship between this physical parameter and the eye irritation potential.



This result is confirmed in Figure 8, which focuses on the correlation between the maximum average score (MAS) and the pH of the test solutions. The correlation coefficient is quite low ($R^2 = 0.22$), and the regression line is not significant (p>0.1).

Further confirmation of the lack of effect of pH on the eye irritation potential of the test surfactants comes from a comparison of the results with lauramine oxide: test solutions were adjusted to two pH values, 7.0 and 10.5. The latter value was the most alkaline pH tested while the other value is very close to natural (physiological) pH values. As reported in Table 16, however, there is very little difference between the two materials in terms of maximum average score, and the solution with the more alkaline pH had the fewer median days to clear. Consequently, the pH range of the test solutions examined in this study did not have a significant effect on the eye irritation scores of the nonionic surfactants tested.

Physical and S	iructural r	opernes	Correlateu	with Eye I	est beures
Nonionic Surfactant ²	MAS ³	pH⁴	HLB ^{5, 6}	Alkyl Chain Length ⁶	EO Units ^{6, 7}
A ₁₂₋₁₃ EO ₃	0.0	5.1	7.9	12.5	2.9
Sorbitan trioleate-EO ₂₀	0.7	7.4	11.0	NA ⁸	20
A ₁₂ EO ₂₃	1.3	3.1	16.9	12.0	23
A ₁₂₋₁₄ EO ₇	4.7	5.8	12	13.6	7.0
A ₁₂₋₁₃ EO _{6.5}	7.0	5.4	12.1	12.5	6.7
Cocamide DEA	7.3	10.0	8.8	12.8	NA
$\mathbf{A}_{12-16} - \mathbf{glucose}_{1.6}$	8.7	9.0	12.1	12.8	NA
A ₈₋₁₀ EO ₅	11.3	6.0	12.0	9.2	4.8
Lauramine oxide (pH=7.0)	11.7	7.0	5.2	12	NA
Lauramine oxide (pH=10.5)	13.0	10.5	5.2	12	NA
Nonylphenol- EO ₉	13.3	6.1	12.9	NA	9.0

Table 19 and Structural Properties Correlated with Eve Test Scores¹ ы

¹ Summary of physical and structural properties to be examined for correlations to maximum average score data from the low volume eye test.

² See Table 10 for full description of nonionics.

³ Maximum average scores (MAS) from Table 16.

⁴ Values from page 9 of Final Report by Steven M. Glaza of Hazleton-Wisconsin in Appendix 3. ⁵ Hydrophillic-lipophilic balance. ⁶ Data from Table 10.

⁷ Oxyethylene units.

⁸ Not applicable.



A possible correlation between the hydrophilic-lipophilic balance (HLB) and the maximum average score of the nonionic surfactants tested is examined in Figure 9. The linear regression line is not significant. No clear relationship between HLB and MAS is evident in this plot.

However, if one focuses on the alcohol ethoxylates, the only set of structurally homologous materials among the nonionic surfactants tested, a somewhat clearer pattern is observed (Figure 10). The data indicate that the three alcohol ethoxylates having an HLB of approximately 12 have a higher eye irritation potential than the two alcohol ethoxylates which have either lower or higher HLB values. These results support and confirm the hypothesis previously discussed (under "Structure-Activity Relationships Among Alcohol Ethoxylates" above) that alcohol ethoxylates with approximately 60% EO (i.e. HLB = 12) have a higher eye irritation potential than alcohol ethoxylates with either lower higher weight % EO (i.e. lower or higher HLB values). Consequently, the results of this study confirm the utility of HLB and weight percent EO in predicting the relative eye irritation potential of this major class of nonionic surfactants.

It is also evident that HLB in itself is not the only factor which predicts relative eye irritation potential since the maximum average scores of the three alcohol ethoxylates with HLB values close to 12 are significantly different (see Table 18). Among the three alcohol ethoxylates with HLBs of 12, eye irritation potential increases with shorter alcohol chain length. The failure of HLB to predict the eye irritation potential of the other nonionic surfactants examined (Figure 9) indicates that other structural or surfactant properties are important in predicting the eye irritation potential of nonionic surfactants.



Figure 10 HLB vs. MAS Alcohol Ethoxylates Tested by SDA Maximum Average Score, Low Volume Eye Test



Possible correlations between average alkyl chain lengths and maximum average scores, and between the average oxyethylene units and MAS, of the nonionic surfactants tested are examined in Figures 11 and 12, respectively. The linear regression lines are not significant. No clear relationship between alkyl chain length or oxyethylene units and MAS is evident in these plots. Fred Heitfeld (Appendix 8) reached a similar conclusion ("the data appear random") in his comparison of the effect on irritancy of alkyl chain length and the degree of ethoxylation for the straight chain nonionics.



Results of Equilibrium Surface Tension Measurements

Surfactant Effectiveness: Dewey Smith of CONDEA Vista reported (Appendix 5, letter of October 28, 1994) that the test solutions of TWEEN 85 (sorbitan trioleate-EO₂₀), cocamide DEA and GLUCOPON 625CS (A_{12-16} - glucose_{1.6}) were highly viscous and had to be diluted with water for an accurate measurement of equilibrium (static) surface tension. Dilution with water is a valid procedure for reducing the viscosity without affecting the surface tension as long as the test solutions remain at concentrations above their critical micelle concentrations. Mr. Smith conducted the proper control experiment, showing (Table 20) that a further dilution of these test materials did not reduce the surface tension measurements. These results demonstrate the validity of the original dilution of the test materials for surface tension measurements.



Table 20 Control Experiment: Unchanged Surface Tension on Further Dilution¹

Nonionic Surfactant ²	15 mL sample + 45 mL water ³	15 mL sample + 50 mL water ⁴
Sorbitan trioleate-EO ₂₀	32.81	32.97
Cocamide DEA	26.79	26.70
A_{12-16} -glucose _{1.6}	28.63	28.30

¹ Surface tension measurements (in dynes/cm) on dilutions of test solutions of nonionic surfactants. CONDEA Vista data (See Appendix 5).

³ 1:3 dilution.

² See Table 10 for full description of nonionics. ⁴ 3:10 dilution.

The CONDEA Vista results from surface tension measurements on the test solutions are shown in Table 21. As expected, all of the surfactant solutions tested were surface active, substantially reducing the surface tension of the test solutions compared to water, which has a surface tension of approximately 73 dynes/cm at room temperature. Surface tension values for the test solutions range

from a low of 25.6 dynes/cm for $A_{12-13}EO_3$ to a high of 41.4 dynes/cm for $A_{12}EO_{23}$. Based on this data, $A_{12-13}EO_3$ is the most <u>effective</u> nonionic surfactant tested since it produces the largest reduction of surface tension while $A_{12}EO_{23}$ is the least effective.

An additional set of equilibrium surface tension measurements were determined by United States Testing Company, Inc. U.S. Testing also noted (Appendix 6, report dated 2/1/95, pages 3-4) that sample 7 (sorbitan trioleate-EO₂₀) and sample 10 (cocamide DEA) were more viscous than the other samples but did not dilute samples for surface tension measurements. U.S. Testing did not comment on the viscosity of A₁₂₋₁₆-glucose_{1.6}.

Surface	l'ension Me	asurements	from Thre	e Laborator	ies
Nonionic Surfactant ²	MAS ³	CONDEA- Vista data⁴	U.S. Testing data ⁵	Sensa- Dyne data ⁶	Average ⁷
A ₁₂₋₁₃ EO ₃	0.0	25.56	24.4	30.4	26.79
Sorbitan trioleate-EO ₂₀	0.7	32.818	39.1	52.1	41.34
A ₁₂ EO ₂₃	1.3	41.39	38.5	43.3	41.06
A ₁₂₋₁₄ EO ₇	4.7	28.29	27.2	30.1	28.53
A ₁₂₋₁₃ EO _{6.5}	7.0	27.46	26.3	28.4	27.39
Cocamide DEA	7.3	26.79 ⁸	29.5	29.3	28.53
$\begin{array}{c} \mathbf{A}_{12\text{-}16} \text{ -}\\ \mathbf{glucose}_{1.6} \end{array}$	8.7	28.63 ⁸	28.9	30.8	29.44
A ₈₋₁₀ EO ₅	11.3	26.43	25.4	25.2	25.68
Lauramine oxide (pH=7.0)	11.7	31.92	31.2	33.7	32.27
Lauramine oxide (pH=10.5)	13.0	32.54	31.4	33.3	32.41
Nonylphenol- EO ₉	13.3	31.33	30.4	32.4	31.38

Table 21Surface Tension Measurements from Three Laboratories1

¹ Summary of surface tension effectiveness measurements on nonionic surfactant test solutions.

² See Table 10 for full description of nonionics.

⁴ See Appendix 5 for data.

⁶ See Appendix 7 for data.

³ Maximum average scores (MAS) from Table 16.

⁵ See Appendix 6 for data.

⁸ Based on 1:3 dilution, see Table 20.

⁷ Average of three surface tension measurements.

As summarized in Table 21, the surface tension measurements reported by U.S. Testing cover a range of values from 24.4 dynes/cm to 39.5 dynes/cm, almost identical to that reported by CONDEA Vista. However, the surfactant producing the lowest surface tension, $A_{8-10}EO_5$, and the surfactant producing the highest surface tension, sorbitan trioleate-EO₂₀, are different from those in the CONDEA Vista data.

SensaDyne Corporation also estimated equilibrium surface tension values for each surfactant solution (Fax from T.C. Christensen, dated March 2, 1995, Appendix 7). The SensaDyne reports do not contain any remarks about the viscosity of the surfactant solutions. However, SensaDyne diluted the test solutions by adding small aliquots (400 μ L) of the surfactant solution to a large volume of water (100 mL) and measuring the decrease in surface tension as additional amounts of surfactant were added. This is a very different procedure from that used by CONDEA Vista and U.S. Testing for measuring surface tension.

The surface tension measurements reported by SensaDyne Corporation are summarized in Table 21. Surface tension values range from 25.2 to 52.1 dynes/cm. Other than the very high value for sorbitan trioleate- EO_{20} (52.1), this range is very similar to those reported by CONDEA Vista and U.S. Testing.

The surface tension values estimated by the three laboratories are compared in Figure 13. In this figure the surfactants are rank ordered from 1 to 11 based on their MAS scores, as listed in Table 21. As can been seen, the surface tension values measured by the three laboratories are very similar except for sorbitan trioleate- EO_{20} (surfactant #2 in the figure). Interestingly, the test solution of this surfactant was reported to be viscous by both CONDEA Vista and U.S. Testing. The test solution was diluted one-to-three with water by CONDEA Vista to reduce the viscosity without reducing the surface tension. The surface tension value measured by CONDEA Vista was lower than that reported by U.S. Testing and SensaDyne, suggesting that the viscosity of the solution may have contributed to the higher surface tension values reported by U.S. Testing and SensaDyne.

High viscosities were also reported for cocamide DEA (by CONDEA Vista and by U.S. Testing) and for A_{12-16} - glucose_{1.6} (by CONDEA Vista). These two materials were also diluted by CONDEA Vista before testing. As shown in Table 21, the CONDEA Vista values for surface tension for these two surfactants are lower that the values reported by the other two laboratories. However, the differences are small and similar to the differences in values reported by the three laboratories for other surfactants in Table 21. Apparently only in the case of sorbitan trioleate-EO₂₀ did the viscosity of the surfactant solution noticeable affect the surface tension values measured by the three laboratories.

As shown in Figure 13, the surface tension values reported by SensaDyne tend to be slightly higher than those reported by CONDEA Vista and U.S. Testing. As noted previously, however, the

differences between the values reported by the three laboratories tend to be small (except for the results with sorbitan trioleate- EO_{20}). The average of the surface tension values determined by the three laboratories for each surfactant has been calculated and these are shown in Table 21.



Comparing the reported values for surface tension effectiveness from Table 12 for $A_{12-13}EO_{6.5}$ (28 dynes/cm), A_{12-16} - glucose $_{1.6}$ (29.3 dynes/cm) and nonylphenol-EO $_{9}$ (30 dynes/cm) with the measured values in Table 21 indicates that all testing laboratories were able to generate values similar, but not identical, to the reported values. Differences in apparatus, experimental conditions and technique probably account for the small differences observed.

Surfactant Efficiency and CMC Values: The efficiency is the amount of surfactant required to reduce the surface tension of water by 20 units (i.e., from 73 to 53 dynes/cm) while the critical micelle concentration is the amount of surfactant required to produce surfactant micelles. Efficiency and CMC values for the surfactants can be determined from the SynsaDyne Corporation data because SynsaDyne diluted each surfactant solution and measured surface tensions at increasing surfactant concentrations. Graphs of surface tension verses concentration for each surfactant are included in the report from Tanya Christensen of SynsaDyne to estimate the critical micelle concentration for each surfactant at each bubble rate. Basically this procedure entails estimating the break point in the surface tension verses concentration curves, since the break in the curve is caused by micelle formation.

SynsaDyne Corporation considered the CMC values determined at the slowest bubble rate (one bubble per 10 seconds) to be the equilibrium CMC. As discussed above under "Effectiveness," there is good agreement between the surface tension values at the CMC measured by SynsaDyne and those estimated by CONDEA Vista and U.S. Testing Company using a different test method. The similarities of the CMC values supports SynsaDyne's conclusion that the surface tension parameters measured at the slowest bubble rate were determined under equilibrium conditions.

Volumes of surfactant solution ("saturation concentration") required to achieve the lowest surface tension are summarized in the report from SynsaDyne Corporation dated March 2, 1995 (Appendix 7). Saturation concentrations were converted into nominal CMC values (in units of moles of surfactant per 100 mL water) in the SensaDyne report of March 21, 1995 (Appendix 7), but these values are in error. Consequently, CMC values (in molar concentration units) were calculated directly from the saturation concentrations using the actual surfactant concentrations (Table 15). The results from these calculations are shown in Table 22. For comparison, the maximum average Draize scores and the surface tension values at the CMC are included in the table.

The graphs used by SynsaDyne to estimate CMC values (T. Christensen, report dated March 21 1995, Appendix 7) were examined to determine if they could be used to estimate surfactant efficiency. For all the surfactants except three (sorbitan trioleate- EO_{20} , lauramine oxide, pH 10.5 and lauramine oxide, pH 7.0) the smallest increment of surfactant solution added (0.4 mL per 100 mL of water) reduced the surface tension of water to considerably below 53 dynes/cm. Consequently, the volume of surfactant solution required to reduce the surface tension of water by exactly 20 units was calculated from the raw data (Appendix 7) using simple proportion. The volume estimated was then converted to a molar concentration of surfactant using the actual concentration of surfactant in the solution (Table 15). These results are also shown in Table 22.

Figure 14 shows a comparison of the equilibrium surface tension data measured by SynsaDyne Corporation. To facilitate visualization of the data, CMC was plotted in 0.5 mM units, i.e. the values in Figure 14 are double the values in Table 22. Also, efficiency was plotted in 0.1 mM units so that the values in Figure 14 are ten-fold higher than the actual values given in Table 22.

As shown in Figure 14, there is some similarity in the pattern of surfactant values for effectiveness (in dynes/cm) and efficiency (in 0.1 mM units) when plotted on the same numerical scale. This plot suggests that effectiveness (the concentration of surfactant required to reduce the surface tension of water by a fixed amount) and efficiency (the maximum reduction in the surface tension of water produced by the surfactant) are related for this group of nonionic surfactants. Apparent exceptions to the pattern are surfactants # 3 ($A_{12}EO_{23}$) and #11 (nonylphenol-EO₉), both of which are relatively more efficient than the other nonionic surfactants than predicted from the relative effectiveness of the surfactants.

Synsadyne Equinibitum Surface Tension Treusurenes					
Nonionic Surfactant ²	MAS ³	Effectiveness⁴ (dynes/cm)	CMC ⁵ (mM)	Efficiency ⁶ (mM)	
A ₁₂₋₁₃ EO ₃	0.0	30.4 a	9.7 cde	0.49 a	
Sorbitan trioleate-EO ₂₀	0.7	52.1	6.7 abc	5.34 b	
A ₁₂ EO ₂₃	1.3	43.3	4.4 a	0.46 a	
A ₁₂₋₁₄ EO ₇	4.7	30.1 a	6.2 abc	0.43 a	
A ₁₂₋₁₃ EO ₆₅	7.0	28.4	7.3 abcd	0.41 a	
Cocamide DEA	7.3	29.3	11.6 e	0.58 a	
$\begin{array}{c} \mathbf{A}_{12-16} \text{ -}\\ \mathbf{glucose}_{1.6} \end{array}$	8.7	30.8 a	10.6 de	0.57 a	
$A_{8-10}EO_5$	11.3	25.2	9.4 cde	0.44 a	
Lauramine oxide (pH=7.0)	11.7	33.7 b	8.4 bcde	1.36 a	
Lauramine oxide (pH=10.5)	13.0	33.3 b	8.4 bcde	1.90 ab	
Nonylphenol- EO ₂	13.3	32.4	5.4 ab	0.44 a	

Table 22SynsaDyne Equilibrium Surface Tension Measurements1

¹ Values followed by the same letter are not significantly different for that surfactant property. See "Statistical Analysis of Surface Tension Results."

² See Table 10 for full description of nonionics. ³ Maximum average scores (MAS) from Table 16.

⁴ Minimum surface tension produced (at CMC). See Appendix 7 (report of March 2, 1995) for data.

⁵ Critical micelle concentration (in millimoles per liter). Calculated from saturation concentrations (report of March 2, 1995) and actual surfactant concentrations (Table 15). Saturation concentrations were estimated only to the nearest 0.4 mL since surfactant test solutions were added to 100 mL of water in 400- μ L aliquots. ⁶ Concentration to reduce surface tension of water exactly 20 dynes/cm. Calculated from the raw data

in Appendix 7 by simple proportion using the two volumes of surfactant solution giving surface tension values immediately above and below 53 dynes/cm, the approximate surface tension of water reduced by 20 units. For most surfactants, these volumes were 0 mL and 0.4 mL. The volumes of surfactant solution estimated to reduce the surface tension of water by 20 dynes/cm were converted to surfactant concentrations (in millimoles per liter) using the concentrations of surfactants in the test solutions shown in Table 15.



There is no apparent relationship between CMC and effectiveness or efficiency in Figure 14. Consequently, correlations to surfactant properties of nonionics should consider CMC as well as efficiency/effectiveness.

Comparing the reported values for equilibrium CMC from Table 12 for $A_{12-13}EO_{6.5}$ (0.035 mM), A_{12-16} - glucose_{1.6} (0.066 mM) and nonylphenol-EO₉ (0.160 mM) and with the measured CMC values in Table 22 reveals that the SynsaDyne values are approximately 100-fold higher than the reported values. Similarly, comparing the reported values for equilibrium surface tension efficiency from Table 12 for $A_{12-13}EO_{6.5}$ (0.00015 mM), A_{12-16} - glucose_{1.6} (<0.012 mM) and nonylphenol-EO₉ (<0.0091 mM) and with the measured efficiency values in Table 22 indicates that the SynsaDyne values are 50 to 2700-fold higher than the reported values. These large differences are not consistent with SynsaDyne's contention that the surface tension parameters measured at the slowest bubble rate (1 bubble per 10 seconds) were determined under equilibrium conditions. If equilibrium CMC or efficiency are relevant to predicting the eye irritation potential of nonionic surfactants, then these values should be redetermined using conventional equilibrium techniques.

Results of Dynamic Surface Tension Measurements

A major advantage of the SynsaDyne technology for measuring surface tension is that it allows the measurement of surface tension parameters under dynamic conditions, i.e. in the time period before

equilibrium conditions are established. Dynamic surface tension measurements have proven to be useful in a number of applications, such as printing and coating, where the speed of the surfactant in lowering surface tension is important (see Appendix 7).

SynsaDyne Corporation measured the surface tension as function of surfactant concentration under three dynamic conditions: 0.24, 1 and 4 bubbles per second). This analysis will focus on the surface tension values at the highest bubble rate used (4 bubbles per second). If speed of the surfactant is an important parameter in predicting eye irritation potential, then any correlations to surface tension values should be most obvious under those conditions.

Surface tension values (effectiveness) at the "saturation concentration" (critical micelle concentration) are summarized in the report from Ms. Christensen of SensaDyne Corporation dated March 21, 1995 (Appendix 7). This report also contains an estimate of the moles of surfactant at the CMC, but these values are in error. Consequently, CMC values were calculated from the saturation concentrations listed in the report, correcting for the actual concentrations of the surfactant test solutions given in Table 15. The values for dynamic surface tension effectiveness and CMC are summarized in Table 23, along with the maximum average scores (MAS) in the low volume eye test.

The efficiency, the concentration of surfactant required to reduce the surface tension of water by exactly 20 units, was estimated from the raw data using simple proportion to calculate the volume of surfactant solution required. For all surfactants but two, the data used were the two volumes of surfactant solution which gave surface tension readings immediately above and below the surface tension value of water reduced by exactly 20 units. For two surfactants ($A_{12}EO_{23}$, and sorbitan trioleate- EO_{20}), the maximum surface tension reduction, observed at the CMC, was less than 20 dynes/cm (see Table 23). Consequently, the efficiency of these two surfactants cannot be calculated. Instead, the CMC values for these two surfactants was used as an estimated of the efficiency. For the remaining surfactants, the volume estimated to reduce the surface tension of water by exactly 20 units was then converted to a molar concentration of surfactant using the actual concentration of surfactant in the test solution shown in Table 15. The estimates for dynamic surface tension efficiency are summarized in Table 23.

Figure 15 shows a comparison of the dynamic surface tension measurements on the nonionic surfactants as determined at the fastest bubble rate (4 bubbles per second). It should be noted that the efficiency values for rank order surfactants #1 ($A_{12-13}EO_3$) and #2 (sorbitan trioleate- EO_{20}) were plotted as the CMC values since the efficiencies could not be determined.

There is some similarity between the shapes of the three curves suggesting a relationship between effectiveness, CMC and efficiency under these dynamic conditions. However, there are a number of exceptions to this pattern. For instance, rank order surfactant #2 (sorbitan trioleate- EO_{20}) has a lower relative CMC than predicted from its relative effectiveness. Rank order surfactants #3 ($A_{12}EO_{23}$) and #11 (nonylphenol- EO_9) have higher relative effectiveness values than predicted from their relative CMC and efficiency values. Surfactant #7 (A_{12-16} - glucose_{1.6}) has a relatively lower

Synsadyne Dynamic Surface Tension Measurements					
Nonionic Surfactant ²	MAS ³	Effectiveness ⁴ (dynes/cm)	CMC ⁵ (mM)	Efficiency ⁶ (mM)	
A ₁₂₋₁₃ EO ₃	0.0	53.8	19.2	>19.2	
Sorbitan trioleate-EO ₂₀	0.7	69.8	5.1	>>5.17	
A ₁₂ EO ₂₃	1.3	46.5 a	9.0 a	0.56 a	
A ₁₂₋₁₄ EO ₇	4.7	41.9	19.5 de	2.92 ab	
A ₁₂₋₁₃ EO _{6.5}	7.0	36.6	16.6 cd	1.36 ab	
Cocamide DEA	7.3	39.1	22.0 e	5.0 b	
A_{12-16} - glucose _{1.6}	8.7	46.9 a	20.0 de	9.0	
A ₈₋₁₀ EO ₅	11.3	27.5	10.2 ab	0.84 a	
Lauramine oxide (pH=7.0)	11.7	35.3 b	22.7 e	3.4 ab	
Lauramine oxide (pH=10.5)	13.0	35.6 b	22.1 e	3.1 ab	
Nonylphenol- EO ₉	13.3	35.6 b	13.4 bc	0.77 ab	

 Table 23

 SynsaDyne Dynamic Surface Tension Measurements¹

¹ Values were determined at the maximal bubble speed tested, 4 bubbles per second. Surface tension values determined under these conditions are the furthest from equilibrium conditions, shown in Table 22, where the bubble rate was 0.1 bubble per second. Values followed by the same letter are not significantly different for that surfactant property. See "Statistical Analysis of Surface Tension Results."

² See Table 10 for full description of nonionics. ³ Maximum average scores (MAS) from Table 16.

⁴ Minimum surface tension produced (at CMC). See Appendix 7 (report of March 21, 1995) for data.

⁵ Critical micelle concentration (in millimoles per liter). Calculated from saturation concentrations (report of March 21, 1995) and actual surfactant concentrations (Table 15). Saturation concentrations were estimated only to the nearest 0.4 mL since surfactant test solutions were added to 100 mL of water in 400-μL aliquots. ⁶ Concentration to reduce surface tension of water exactly 20 dynes/cm. Calculated from the raw data in Appendix 7 by simple proportion using the two volumes of surfactant solution giving surface tension values immediately above and below 53 dynes/cm, the approximate surface tension of water reduced by 20

units. Two surfactants ($A_{12}EO_{23}$, and sorbitan trioleate- EO_{20}) had surface tension values above 53 dynes/cm at the CMC (concentration for minimal surface tension) and the volume of surfactant at the CMC was used as an estimation of the efficiency. The volumes of surfactant solution estimated to reduce the surface tension of water by 20 dynes/cm were converted to surfactant concentrations (in millimoles per liter) using the concentrations of surfactants in the test solutions shown in Table 15.

⁷ Indicates concentration of surfactant required to reduce surface tension of water by 20 dynes/cm is "much greater" than 5.1 mM. Compare surface tension at CMC to surface tension of water, approximately 73 dynes/cm.

CMC value than predicted from its relative effectiveness and efficiency. Because of these exceptions, correlations to dynamic surface tension properties should consider effectiveness, CMC and efficiency.



As shown in Table 23, values for dynamic surface tension effectiveness range from 27.5 to 69.8 dynes/cm, a somewhat larger range of values than those observed for static surface tension (25.2 to 52.1 dynes/cm, Table 22). Similarly, the values for dynamic surface tension CMC (5.1 to 22.7 mM) and efficiency (0.56 to 19.2 mM) cover a wider range than the values for equilibrium surface tension CMC (4.4 to 11.6 mM) and efficiency (0.41 to 5.34 mM). Use of dynamic conditions gives more separation of surfactant values than equilibrium conditions because an additional surfactant parameter is measured, namely the speed at which surfactant molecules diffuse through the surfactant solution to the interface and align at the interface to reduce surface tension.

Note that the effectiveness (the surface tension at the CMC) of the surfactants measured under dynamic conditions is reduced (higher surface tension values observed) compared to those measured under static conditions. This is illustrated in Figure 16. Reduced effectiveness is expected under dynamic conditions because there is less time for the surfactants to move to the air-water interface on the bubbles and become optimally aligned to reduce the surface tension.



For five of the nonionic surfactants (Figure 16) there is only a small losses in effectiveness under dynamic conditions (4 bubbles per second) compared to equilibrium conditions (0.1 bubble per second). Although the time available for surfactant diffusion and orientation at the interface decreases 40-fold in going from the slowest bubble rate to the fastest, surfactant effectiveness decreases (the surface tension at the CMC increases) 3.2 dynes/cm (10%) or less.

Figure 17 compares the critical micelle concentration observed under dynamic conditions with that those measured at equilibrium. As can be seen, CMCs are generally, but not always, higher under dynamic conditions. The dynamic situation is more complex with CMCs than with effectiveness because there is a 30 second mixing period after addition of each aliquot of surfactant before surface tension is measured. This period allows surfactant micelles to form if the surfactant concentration is high enough, i.e. above the equilibrium CMC. However, when bubbles are formed in the surfactant solution (to measure dynamic surface tension), surfactant molecules move to the new interfaces formed, causing some micelles to break apart (and others to reform) as the solution is depleted of surfactant molecules. Generally surfactant diffusion through the solvent (water) is the slowest step in this process (Myers, 1991). As the bubble rate increases, the time available for surfactant diffusion is decreased. However, a higher surfactant concentration can somewhat compensate for this reduced time and so the apparent CMC values would be expected to increase. Eventually, as the bubble rate further increases, bulk diffusion is less and less able to compensate for the reduced time available, the surfactant content of fewer micelles is depleted until the apparent CMC drops to equilibrium CMC values.



This pattern of increasing CMC as the bubble rate increases is seen with all the nonionic surfactants tested, as shown in Table 24. However, for two nonionics (sorbitan trioleate- EO_{20} , and $A_{8-10}EO_5$), the apparent CMCs decrease at the highest bubble rate tested (4 bubbles per second) to values close to the equilibrium CMCs.

Surfactant efficiencies under equilibrium and dynamic conditions are compared directly in Figure 18. Higher surfactant concentrations are required under dynamic compared to equilibrium conditions to reduce the surface tension of water by 20 units. Reduced efficiencies are expected under dynamic conditions because there is less time for diffusion of surfactant to the interface and consequently, higher surfactant concentrations are required to produce the same surface tension reductions in water.

What is somewhat unexpected is the relatively small losses in efficiencies observed for some of the surfactants under dynamic conditions (4 bubbles per second) compared to equilibrium conditions (0.1 bubble per second). Although the time available for surfactant diffusion and orientation at the interface decreases 40-fold in going from the slowest bubble rate to the fastest, surfactant efficiency decrease 2.5-fold or less for six of the surfactant solutions.

Nonionic Surfactant ²	CMC At Slowest Bubble Rate ³	CMC At Slow Bubble Rate ⁴	CMC At Medium Bubble Rate ⁵	CMC At Fastest Bubble Rate ⁶
A ₁₂₋₁₃ EO ₃	9.7	14.6	18.6	19.2
Sorbitan trioleate-EO ₂₀	6.7	7.1	8.2	5.1
A ₁₂ EO ₂₃	4.4	6.5	6.5	9.0
A ₁₂₋₁₄ EO ₇	6.2	8.4	15.5	19.5
A ₁₂₋₁₃ EO _{6.5}	7.3	8.7	15.4	16.6
Cocamide DEA	11.6	18.0	17.3	22.0
$\begin{array}{c} A_{12-16} - \\ glucose_{1.6} \end{array}$	10.6	16.8	18.7	20.0
A ₈₋₁₀ EO ₅	9.4	8.7	13.8	10.2
Lauramine oxide (pH=7.0)	8.4	12.7	16.8	22.7
Lauramine oxide (pH=10.5)	8.4	13.4	16.8	22.1
Nonylphenol- EO ₉	5.4	6.9	12.0	13.4

Table 24Critical Micelle Concentrations of Nonionic Surfactants vs. Bubble Rate1

¹ Critical micelle concentrations (CMC) were calculated from saturation concentrations (SynsaDyne reports of March 2, and March 21, 1995, Appendix 7) and actual surfactant concentrations (Table 15). Saturation concentrations were estimated only to the nearest 0.4 mL since surfactant test solutions were added to 100 mL of water in $400-\mu$ L aliquots.

² See Table 10 for full description of nonionics.

³ 0.1 bubbles per second.

⁵ 1 bubble per second.

⁴ 0.24 bubbles per second.
⁶ 4 bubbles per second.

61



Results of Interfacial Surface Tension Measurements

Interfacial surface tension can be measured at a liquid-liquid interface, such as that formed by water and a water-immiscible organic liquid. Interfacial surface tension measurements on the surfactants, using octanol as the organic liquid, were conducted by United States Testing Company, Inc. The company noted (Appendix 6, report dated 2/1/95, page 2) that samples 1 (A₁₂₋₁₃EO_{6.5}), 2 (A₁₂₋₁₄EO₇), 4 (A₁₂₋₁₃EO₃), 7 (sorbitan trioleate-EO₂₀) and 10 (cocamide DEA) formed a white substance at the interface when layered with octanol. This material seem to be a precipitate, indicating that the test solution concentrations greatly exceeded their octanol solubility. It is not know if this material would interfere with the measurement of interfacial surface tension but U.S. Testing made no mention of interference with the measurements of interfacial surface tension.

The results of the interfacial surface tension measurements are summarized in Table 25. Values range from a low of 0.0 (for $A_{12-13}EO_3$, $A_{12-14}EO_7$, $A_{12-13}EO_{6.5}$, and nonylphenol-EO₉) to a high of 8.2 for cocamide DEA. The range of surface tension values measured with this method seems rather narrow with four surfactants (noted above) giving the same measurement (0.0).

Nonionic Surfactant ²	MAS ³	Equilibrium Surface Tension ^{4,6}	Interfacial Tension ^{4, 6}	Contact Angle ^{5, 6}
A ₁₂₋₁₃ EO ₃	0.0	24.4 (0.06)	0.0 (0.0)	23.0 (3.0)
Sorbitan trioleate-EO ₂₀	0.7	39.1 (0.76)	6.16 (0.08)	79.0 (3.5)
A ₁₂ EO ₂₃	1.3	38.5 (0.04)	0.8 (0.04)	87.0 (1.8) a ⁷
A ₁₂₋₁₄ EO ₇	4.7	27.2 (0.05)	0.0 (0.0)	47.0 (0.6)
A ₁₂₋₁₃ EO _{6.5}	7.0	26.3 (0.06)	0.0 (0.0)	36.0 (4.8) c
Cocamide DEA	7.3	29.5 (0.53)	8.2 (.24)	67.0 (4.5)
$\begin{array}{c} \mathbf{A}_{12-16} -\\ \mathbf{glucose}_{1.6} \end{array}$	8.7	28.9 (0.28)	1.3 (0.35)	86.0 (1.3) a
A ₈₋₁₀ EO ₅	11.3	25.4 (0.06)	1.5 (0.13)	32.0 (5.5) c
Lauramine oxide (pH=7.0)	11.7	31.2 (0.05)	2.6 (0.07)	39.0 (5.5) c
Lauramine oxide (pH=10.5)	13.0	31.4 (0.07)	4.1 (0.06)	53.0 (3.4) b
Nonylphenol- EO ₉	13.3	30.4 (0.05)	0.0 (0.0)	56.0 (3.8) b

Table 25Surfactant Properties Measured by United States Testing Company, Inc.1

¹ See Appendix 6 for data.

² See Table 10 for full description of nonionics.

³ Maximum average scores (MAS) from Table 16.

⁴ Dynes/cm. ⁵ Degrees.

⁶ Numbers in parentheses are the \pm values for the 95% confidence interval (C.I.), calculated from the standard deviation (s.d.) and the number (n) of replicates (10) and using the t-value of 2.262 for 9 degrees of freedom, where 95% C.I. = t-value x s.d. / square root{n}.

⁷ Values followed by the same letter are not significantly different.

Interfacial and equilibrium surface tension values measured by U.S. Testing Company are compared in Figure 19. There seems to be a similar pattern of response between the surfactant solutions for interfacial and equilibrium surface tension. One possible exception to this pattern is rank order surfactant #11 (nonylphenol-EO₉) which appears to have a lower than expected interfacial surface tension based on its equilibrium surface tension. Surfactant #3 ($A_{12}EO_{23}$) and #7 (A_{12-16} - glucose_{1.6})

also appear to give somewhat lower interfacial surface tension values than predicted based on their equilibrium surface tension values. However, the narrow range of values observed for interfacial surface tension makes such interpretations somewhat uncertain.



Results of Contact Angle Measurements

The surface tension of a surfactant solution can also be determined by measuring the contact angle formed by a drop of the surfactant solution on a flat surface (see Figure 6). Contact angle measurements on the surfactant solutions, using TELFON tape for the surface, were conducted by U.S. Testing Company. The laboratory noted (Appendix 6, report dated 2/1/95, page 4) that sample 7 (sorbitan trioleate-EO₂₀) and sample 10 (cocamide DEA) were more viscous than the other samples and that sample 10 (cocamide DEA) contained numerous air bubbles which could not be eliminated. There was no indication that the viscosity or the air bubbles interfered with measurement of contact angle.

The results from the contact angle measurements are summarized in Table 25 along with the results from equilibrium and interfacial surface tension measurements. Contact angle values range from a low of 23 degrees for $A_{12-13}EO_3$ to a high of 87 degrees for $A_{12}EO_{23}$. Lowest contact angles (flattened bubbles) are produced by solutions having the lowest surface tension while highest contact angles (rounded bubbles) by solutions having the highest contact angle (see Figure 6).

Consequently, contact angle values vary in the same direction as equilibrium and interfacial surface tension values although the units (degrees for contact angle, dynes/cm for equilibrium and interfacial surface tension) differ.

Contact angle values are compared to interfacial and equilibrium surface tension values, all determined by U.S. Testing Company, in Figure 19. As can be seen, there is a similar pattern of response between the surfactant solutions for contact angle and surface tension. One possible exception to this pattern is rank order surfactant #9 (lauramine oxide, pH=7.0) which appears to have a lower than expected contact angle based on its surface tension. The reason for this difference is not known.

Statistical Analysis of Surface Tension Measurements

Statistical analysis cannot be conducted on the CONDEA Vista equilibrium surface tension data since replicate values were not reported.

United States Testing Company, Inc., reported ten replicate measurements of equilibrium surface tension, interfacial tension and contact angle determinations for each surfactant solution (Appendix 6). U.S. Testing calculated the standard deviation of each measured value as well as the average and corrected values shown in Table 25. The standard deviation and the number of replicate tests can be used to calculate "95% confidence values," the numbers added and subtracted from the average which give the 95% confidence interval, the range of values which are predicted to contain the true numerical result in 95 out of 100 tests. Consequently, tests whose 95% confidence intervals do not overlap are statistically different.

The 95% confidence values (C.V.) can be calculated from the equation, C.V. = $(t-value) \times (s.d.) \div$ square root{n}, where the "t-value" is for 0.05 probability level (two-tailed) at n-1 degrees of freedom, "s.d." is the standard deviation and "n" is the number of replicate tests. For ten tests, the degrees of freedom = 9, the t-value = 2.262 and the square root of n = 3.16. Consequently, the 95% confidence values are smaller than the standard deviation for this data.

The calculated 95% confidence values for the U.S. Testing data are included in Table 25. For equilibrium surface tension, the values are typically less than 0.1 dynes/cm, the smallest incremental value measured. Statistically, all of the values are significantly different.

Confidence values larger than 0.1 dynes/cm were reported for three surfactants: sorbitan trioleate- EO_{20} , cocamide DEA, and A_{12-16} - glucose_{1.6}. These are the three surfactant solutions reported to be viscous ("Results of Equilibrium Surface Tension Measurements"). Apparently, the viscosity of these solutions impacted the reproducibility of the surface tension readings.

The 95% confidence values for the measurement of interfacial tension are also shown in Table 25. Most values are less than 0.1 dynes/cm, the smallest increment measured. Exceptions are for cocamide DEA, A_{12-16} - glucose_{1.6}, and $A_{8-10}EO_5$. The viscosity of the cocamide DEA and A_{12-16} - glucose_{1.6} solutions may have reduced the reproducibility. However, $A_{8-10}EO_5$ was not reported to be viscous or to form a white precipitate when mixed with octyl alcohol (like five other surfactants). Consequently, the reason for the slightly lower reproducibility of this material is not known.

Based on the confidence intervals shown in Table 25, the interfacial tension values for A_{12-16} -glucose_{1.6} (1.3 dynes/cm) and $A_{8-10}EO_5$ (1.5 dynes/cm) are not significantly different, nor, of course, are the four surfactant solutions which gave contact angle values of 0.0 dynes/cm.

Table 25 also includes the 95% confidence values for the measurement of contact angle. Confidence values range from 0.6 to 5.5 degrees with 8 of 11 values in the range of 3.0 to 5.5 degrees. There is no obvious correlation of these values with the contact angle measured, the viscosity of the surfactant solutions or the persistent air bubbles reported in the cocamide DEA solution.

Based on these values for the confidence interval, the contact angle values for $A_{12}EO_{23}$ (87.0 degrees) and A_{12-16} - glucose_{1.6} (86.0 degrees) are not significantly different; nonylphenol-EO₉ (56.0 degrees) and lauramine oxide, pH=10.5 (53.0 degrees) are not significantly different; and lauramine oxide, pH=7.0 (39.0), A_{12-13} -EO_{6.5} (36.0 degrees) and $A_{8-10}EO_5$ (32.0) are not significantly different. This somewhat complex pattern of differences is indicated in Table 25, where contact angle measurements followed by the same letter are not significantly different.

SynsaDyne Corporation reports that surface tension measurements were recorded to the nearest 0.1 dynes/cm reading; consequently, the maximum standard deviation for surface tension measurements was 0.05 dynes/cm. Since all tests were conducted in duplicate, n = 2, degrees of freedom = 1, the t-value for 0.05 probability level (two-tailed) = 12.7, and the 95% confidence value = 0.4 dynes/cm. Based on this confidence value, equilibrium and dynamic surface tension values which are not significantly different are indicated in Tables 22 and Table 23, respectively.

For the measurement of the CMC and of surfactant efficiency, SynsaDyne added 0.4 mL aliquots of surfactant solutions with approximate (see Table 15 for exact values) surfactant concentrations of 0.2 M to 100 mL beakers of water. Consequently, the maximum standard deviation was 0.2 mL x $0.2 \text{ M} \div 100 \text{ mL} = 0.4 \text{ mM}$. Again since all tests were conducted in duplicate, n = 2, degrees of freedom = 1, the t-value for 0.05 probability level (two-tailed) = 12.7, and the 95% confidence value = 3.6 mM. Based on this confidence value, equilibrium and dynamic CMC and efficiency values which are not significantly different are indicated in Tables 22 and Table 23.

The pattern of non-significant differences for equilibrium CMC is quite complex (Table 22) indicating that few values are significantly different. As would be expected from the larger range of values observed for dynamic CMC (and the same confidence values), more significant differences are observed (Table 23). The pattern of non-significant differences is quite simple for equilibrium

efficiency because only one value (for sorbitan trioleate- EO_{20}) is significantly different from the others. Again, better separation was achieved with dynamic efficiency, resulting in more significant differences between surfactants.

Surface Tension Measurement Correlations to Physical and Structural Properties

The physical and structural properties which might predict surface tension measurements include: pH, hydrophilic-lipophilic balance (HLB), alkyl chain length and oxyethylene (EO) units. These physical/structural properties for each surfactant are summarized in Table 19.

pH: Of the physical/structural properties, the pH of the surfactant solutions would not be expected to affect surface tension measurements since the surfactants are nonionic. This hypothesis was examined by conducting linear regression analysis for any correlation between the pH values of the surfactant solutions and the various surface tension measurements reported in Tables 21-25. The results of this analysis are summarized in Table 26. The significance of the correlation coefficients from the regression analysis were determined using the t-tests as previously described under "Structure-Activity Relationships Among Alcohol Ethoxylates." As can be seen, the regression analysis reveals significant linear relationships between pH and: equilibrium CMC, interfacial surface tension, dynamic CMC (bubble rate = 0.24/sec) and dynamic surface tension efficiency. These relations can be examined directly by comparing surface tension measurements determined with the lauramine oxide solutions at pH 7.0 and pH 10.0, where the structure of the surfactant is not a possible confounding factor to the pH of the surfactant solutions. A comparison of the surface tension values for the lauramine oxide solutions at the two pH-value indicates that there is no significant difference in equilibrium CMC values (they are identical, see Table 22), or for dynamic CMC at bubble rate = 0.24/sec (see Table 24 and "Statistical Analysis of Surface Tension Results") or for dynamic surface tension efficiency (see Table 23). While there were significant differences in interfacial surface tension for the two surfactant solutions (see Table 25), the positive slope of the line predicted from the regression analysis (Table 26) is contradicted by the lower interfacial tension value for the lauramine oxide solution with the higher pH. Consequently, this analysis suggests, but does not prove, that the pH values of the surfactant solutions may predict certain surface tension properties of nonionic surfactants. Further testing of nonionic surfactant solutions adjusted to different pH-values would be required to confirm or refute this possibility.

Hydrophilic-Lipophilic Balance (HLB): A second physical property, the HLB, was examined for its ability to predict the surface tension properties of the surfactants. Linear regression analysis was conducted on the HLB values of the surfactants and the various surface tension measurements reported in Tables 21-25. The results of this analysis are summarized in Table 27. The regression analysis reveals significant linear relationships between HLB and CMC values, with the significance of the linear regression increasing with the bubble speed, i.e. as conditions move from equilibrium to more dynamic conditions. This pattern is illustrated in Figure 20. Note that the y-intercept

increases and the slope of the line becomes steeper (increasing negative slope) as the bubble rate increases. The consistency of this trend increases one's confidence that the decrease in CMC-values with increasing HLB is real.

Statistical Analysis Summary, pil values vis Surface reaston								
Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)		
EQUILIBRIUM:								
Effectiveness ²	0.09	11		-				
Effectiveness ³	0.001	11						
Effectiveness ⁴	0.02	11						
Effectiveness: average	0.02	11						
CMC ⁴	0.41	11	2.50	<0.05	0.641	3.62		
Efficiency ⁴	0.07	11						
Interfacial Tension ³	0.48	11	2.85	<0.02	0.863	-3.67		
Contact Angle ³	0.04	11						
DYNAMIC ⁴ :								
Effectiveness	0.006	11						
CMC-slow ⁵	0.46	11	2.76	<0.05	1.27	2.39		
CMC-medium⁵	0.26	11	1.76	0.1				
CMC-fastest⁵	0.25	11	1.45	0.2				
Efficiency	0.45	9	2.38	< 0.05	0.75	-2.25		

 Table 26

 Statistical Analysis Summary: pH Values vs. Surface Tension¹

¹ Linear regression analysis of the pH values of surfactant solutions (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Significance was not calculated for R²-values smaller than 0.25. Slope of line and Y-intercept values are reported only for regression lines with significant (p < 0.05) correlation coefficients.

² CONDEA Vista data. ³ U.S. Testing data.

⁴ SynsaDyne data

⁵ Bubble speed -- see Table 24 for rates.

Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						
Effectiveness ²	0.13	11				
Effectiveness ³	0.06	11				
Effectiveness ⁴	0.04	11				
Effectiveness: average	0.08	11				
CMC ⁴	0.29	11	1.93	0.1		
Efficiency ⁴	0.04	11				
Interfacial Tension ³	0.15	11				
Contact Angle ³	0.20	11				
DYNAMIC ⁴ :						
Effectiveness	0.01	11				
CMC-slow ⁵	0.34	11	2.17	0.05	-0.698	18.4
CMC-medium⁵	0.42	11	2.53	< 0.05	-0.748	22.4
CMC-fastest⁵	0.44	11	2.63	< 0.05	-1.14	28.3
Efficiency	0.08	9				

Table 27Statistical Analysis Summary: HLBs vs. Surface Tension1

¹ Linear regression analysis of the hydrophilic-lipophilic balances (HLBs) of nonionic surfactants (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Significance was not calculated for R²-values smaller than 0.25. Slope of line and Y-intercept values are reported only for regression lines with significant ($p \le 0.05$) correlation coefficients. ² CONDEA Vista data. ³ U.S. Testing data. ⁴ SynsaDyne data

⁵ Bubble speed -- see Table 24 for rates.

As shown in Table 27, there are no significant correlations between HLB and any other surfactant property examined.

When searching for correlations between HLB and maximum average Draize scores ("Results from the Low Volume Eye Test — Correlations to Physical Properties / Structure") it was noted that

focusing on the alcohol ethoxylates allowed a clearer pattern of correlations to be observed. This is likely due to the fact that the alcohol ethoxylates are the only set of structurally homologous materials among the nonionic surfactants tested.



Linear regression analysis of the correlations between HLB and surface tension measurements for the five alcohol ethoxylates are summarized in Table 28. Comparison of the results of this analysis with those for all the nonionic surfactants tested (Table 27) reveals that the correlation coefficients for the linear relationships between HLB and CMC values have increased in focusing just on the alcohol ethoxylates. In three of four cases, the probability value (p) also decreased, indicating increased significance, despite the reduction in the number of data points available on alcohol ethoxylates (n = 5) compared to those available for all the nonionics tested (n = 11).

Moreover, a correlation between HLB and equilibrium surface tension effectiveness is now apparent (Table 28). Two of the three laboratories produced data showing a significant correlation and the average values show a trend toward significance. Pooling the data from the three labs produces a highly significant correlation (p < 0.001).

The regression lines from the analysis of HLB vs. effectiveness are shown in Figure 21. Note that the lines are nearly parallel and differ only slightly in y-intercept. The lines indicate that an increase in HLB increases equilibrium surface tension at the CMC, decreasing effectiveness for alcohol ethoxylates.

Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						
Effectiveness ²	0.78	5	3.30	< 0.05	1.82	7.69
Effectiveness ³	0.80	5	3.45	< 0.05	1.62	8.68
Effectiveness ⁴	0.49	5	1.71	0.2		
Effectiveness: Average	0.69	5	2.60	.08		
Effectiveness: Pooled Data ⁶	0.64	15	5.18	<0.001	1.65	9.76
CMC ⁴	0.73	5	2.82	0.07		
Efficiency ⁴	0.09	5				
Interfacial Tension ³	0.16	5				
Contact Angle ³	0.86	5	4.22	<0.05	7.26	-43.4
DYNAMIC ⁴ :						
Effectiveness	0.04	5				
CMC-slow ⁵	0.83	5	3.85	< 0.05	-0.875	20.0
CMC-medium ⁵	0.92	5	6.08	< 0.01	-1.36	30.6
CMC-fastest ⁵	0.53	5	1.85	0.2		
Efficiency	0.30	4				

 Table 28

 Statistical Analysis Summary: Alcohol Ethoxylate HLBs vs. Surface Tension¹

¹ Linear regression analysis of the hydrophilic-lipophilic balances (HLBs) of alcohol ethoxylates (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Significance was not calculated for R²-values smaller than 0.40. Slope of line and Y-intercept values are reported only for regression lines with significant ($p \le 0.05$) correlation coefficients. ² CONDEA Vista data. ³ U.S. Testing data. ⁴ SynsaDyne data

⁵ Bubble speed -- see Table 24 for rates. ⁶ CONDEA Vista, U.S. Testing and SynsaDyne data.

Given the similarity of the pattern of response of surface tension effectiveness, contact angle and interfacial tension noted in Figure 19, and the strength of the correlation between HLB and

effectiveness in the U.S. Testing Corporation data, it is not surprising that contact angle also shows a significant correlation to HLB (Table 28). What might seem surprising is that interfacial tension does not show a similar correlation. However, as shown in Figure 22, the interfacial tension values for the alcohol ethoxylates are very low (all values are less than 2 dynes/cm, see Table 25), producing a line with essentially zero slope and an intercept of zero. In contrast, the contact angle assurements cover a much broader scale (23 to 87 degrees, see Table 25), producing a line with a steeper slope than the line for surface tension effectiveness.



<u>Alkyl Chain Length</u>: A structural property common to most of the nonionic surfactants tested is the length of the alkyl carbon chain (Table 19). Linear regression analysis was conducted to determine any correlation between alkyl chain length and the surface tension measurements reported in Tables 21-25. The results of the analysis are summarized in Table 29. No significant relationships were found between alkyl chain length and any of the surface tension measurements.

Oxyethylene (EO) Units: A second structural property common to most of the surfactants tested is the number of (EO) units per molecule (Table 19). The results of the linear regression analysis for possible correlations between EO units and surface tension measurements are summarized in Table 30. Equilibrium surface tension effectiveness was highly correlated to the number of EO units. Note that the correlation coefficients are higher, and consequently the p-values more significant, than for the correlation of HLB and equilibrium effectiveness for the alcohol ethoxylates
(Table 28). Since the number of EO units per molecule and the HLB are related, this comparison of correlation coefficients indicates that the correlations of equilibrium effectiveness to HLB are related to the effect of the number of EO units per molecule on the HLB.



As shown in Figure 23, the regression lines for EO units versus equilibrium effectiveness generated from the data of the three laboratories have virtually the same y-intercept but slightly different slopes. The difference in the slopes is apparently due to differences in the surface tension measurements for sorbitan trioleate-EO₂₀, which were previously noted in Table 21. Apparently, the viscosity of this material influenced the surface tension measurements (See "Results of Equilibrium Surface Tension Measurements -- Surfactant Effectiveness") causing the differences in the slopes of the regression lines. Note that the positive slopes of all three lines indicate that, as the number of EO units per molecule increases, the surface tension achieved at the CMC increases and the effectiveness decreases.

As noted previously, the similarity of the pattern of responses for surface tension effectiveness, contact angle and interfacial tension noted in Figure 19, and the strength of the correlation between the number of EO units and effectiveness in the U.S. Testing Corporation data (Table 30), predict that contact angle and interfacial tension should also show a significant correlation to the number of EO units. In fact the contact angle shows the highest correlation coefficient to the number of EO units of any single parameter measured (Table 30). However, the interfacial tension does not show a similar correlation. Like the situation with HLB and the alcohol ethoxylates discussed above, the interfacial tension values for the nonionic surfactants are very low (all values range from 0.0 to 8.2

dynes/cm, see Table 25), producing a line with essentially zero slope and an intercept of zero (See Figure 24). In contrast, the contact angle measurements cover a much broader scale (23 to 87 degrees, see Table 25), producing a line with a steeper slope than the line for surface tension effectiveness.

Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						
Effectiveness ²	0.00003	9				
Effectiveness ³	0.007	9				
Effectiveness ⁴	0.05	9				
Effectiveness: Average	0.01	9				
CMC ⁴	0.008	9				
Efficiency⁴	0.002	9				
Interfacial Tension ³	0.0004	9				
Contact Angle ³	0.08	9				
DYNAMIC ⁴ :						
Effectiveness	0.38	9	2.08	0.08		
CMC-slow ⁵	0.09	9				
CMC-medium⁵	0.08	9				
CMC-fastest⁵	0.34	9	1.91	0.1		
Efficiency	0.20	8				

 Table 29

 Statistical Analysis Summary: Alkyl Chain Length vs. Surface Tension¹

¹ Linear regression analysis of the average alkyl chain length of nonionic surfactants (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Significance was not calculated for R²-values smaller than 0.30. Slope of line and Y-intercept values are reported only for regression lines with significant ($p \le 0.05$) correlation coefficients.

² CONDEA Vista data. ³ U.S. Testing data. ⁴ SynsaDyne data

⁵ Bubble speed -- see Table 24 for rates.

Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						
Effectiveness ²	0.85	7	5.41	<0.01	0.648	23.7
Effectiveness ³	0.97	7	12.3	< 0.001	0.779	22.0
Effectiveness ⁴	0.81	7	4.56	<0.01	1.10	23.0
Effectiveness: Average	0.96	7	11.4	<0.001	0.843	22.9
CMC ⁴	0.51	7	2.26	0.07		
Efficiency ⁴	0.29	7				
Interfacial Tension ³	0.32	7	1.54	0.2		
Contact Angle ³	0.95	7	9.65	< 0.001	3.01	19.9
DYNAMIC ⁴ :						
Effectiveness	0.28	7				
CMC-slow ⁵	0.42	7	1.91	0.1		
CMC-medium⁵	0.90	7	6.71	<0.01	-0.521	18.3
CMC-fastest⁵	0.57	7	2.57	0.05	-0.528	18.8
Efficiency	0.17	5				

Table 30Statistical Analysis Summary: EO Number vs. Surface Tension1

¹ Linear regression analysis of the average number of oxyethylene (EO) units per molecule of nonionic surfactants (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Significance was not calculated for R²-values smaller than 0.30. Slope of line and Y-intercept values are reported only for regression lines with significant ($p \le 0.05$) correlation coefficients. ² CONDEA Vista data. ³ U.S. Testing data. ⁴ SynsaDyne data

⁵ Bubble speed -- see Table 24 for rates.

Table 30 also shows that there is a correlation between the number of EO units and the CMC values, particularly under dynamic conditions. Comparison of these correlation coefficients with those in Table 28 for HLB and alcohol ethoxylates reveals that the latter correlation coefficients are larger and the corresponding p-values more significant. This despite the fact that there are fewer data points for HLB and the alcohol ethoxylates (n = 5) than for EO units and the nonionics tested





76

(n = 7). Since the number of EO units per molecule and the HLB are related, this comparison of correlation coefficients indicates that HLB gives the stronger correlation to CMC values and that the correlations of the CMC values to EO units per molecule are related to the effect of the number of EO units per molecule on the HLB.

Comparison to Predicted Correlations: In previous studies (Cox, 1989, Myers, 1991), the surfactant properties of alcohol ethoxylates have been correlated to structural features of the molecules. As summarized in Table 11, these correlations focused on two structural patterns: 1) alkyl chain length (with constant weight % EO) and 2) the weight percent polyoxyethylene (EO) units (with constant alkyl chain length). Table 31 shows the static surface tension properties of the alcohol ethoxylates that can be grouped by these structural features. Note that the three alcohol ethoxylates in the top half of Table 31 fit the first pattern while the alcohol ethoxylates in the bottom half of the table fit the second pattern. Only one alcohol ethoxylate ($A_{12-13}EO_{6.5}$) is common to both patterns.

Table 31 reveals that, as the alkyl chain lengths increase (with constant weight % EO), the surfactant effectiveness decreases (surface tension at the CMC increases). This pattern of response was observed with the SynsaDyne data alone or the average values from the three laboratories. This is the same pattern of response that Cox (1989) had observed with alcohol ethoxylates.

Also as shown in Table 31, the CMCs decrease as the alkyl chain lengths increase. However, the magnitude of the decrease is small and not significant. Cox (1989) had reported the same pattern of response with changes of a much larger magnitude. For instance, Cox (1989) observed that the CMCs (mM values) decreased by factors of ten in going from a "8-60" (where the first number indicates the alkyl chain length and the second number the weight percent EO) to a "10-60" to a "12-60," and by a factor of five in going from a "12-60" to a "14-60."

Cox (1989) did not report on surfactant efficiency but Meyers (1991) indicates that surfactant efficiency should increase with decreasing CMCs since efficiency is defined as the amount of surfactant required to reach the CMC. However, the efficiency data for the three alcohol ethoxylates in Table 31 are not significantly different nor do they show any trend toward increasing with increasing alkyl chain lengths.

For the three alcohol ethoxylates with increasing weight % EO (constant alkyl chain length), the surfactant effectiveness seems to decrease (the surface tension at the CMC increases) with increasing weight % EO. This pattern is somewhat clearer if one uses the average values from the three laboratories rather than the SynsaDyne data alone. Again, Cox (1989) reported the same pattern.

The CMC values in this set of alcohol ethoxylates seem to be decreasing with increasing weight % EO, although only the extreme values are significantly different. In contrast to these results, Cox (1989) reported that CMC values increase with increasing EO content, with CMC values three-fold higher in a "12-80" compared to a "12-40" ethoxylate. Further, Cox (1989) explains that this pattern

was to be expected since the solubility of the alcohol ethoxylates in water increases with increasing EO content thereby increasing the CMC. Perhaps the small difference in alkyl chain lengths among the alcohol ethoxylates in Table 31 has confounded the expected pattern of response of the CMC values.

Equinorium Surface Tension Properties of Alconol Ethoxylates							
Alcohol Ethoxylate ²	Average Alkyl Chain Length ³	HLB ^{3,4}	Weight % EO ^s	Effective- ness (dynes/cm) ⁶	CMC (mM) ⁷	Efficiency (mM) ⁸	
A ₈₋₁₀ EO ₅	9.2	12.0	60.0	25.2 (25.7)	9.4 a	0.44 a	
A ₁₂₋₁₃ EO _{6.5}	12.5	12.1	60.5	28.4 (27.4)	7.3 a	0.41 a	
A ₁₂₋₁₄ EO _{7.0}	13.6	12.0	60.0	30.1 (28.5)	6.2 a	0.43 a	
			Observed Pattern	Decrease	Decrease	?	
			Predicted Pattern ⁹	Decrease	Decrease	Increase	
A ₁₂₋₁₃ EO ₃	12.5	7.9	39.5	30.4 (26.8)	9.7 a	0.49 a	
A ₁₂₋₁₃ EO _{6.5}	12.5	12.1	60.5	28.4 (27.4)	7.3 a,b	0.41 a	
A ₁₂ EO ₂₃	12.0	16.9	84.5	43.3 (41.1)	4.4 b	0.46 a	
			Observed Pattern	Decrease	Decrease	?	
			Predicted Pattern ⁹	Decrease	Increase	Decrease	

 Table 31

 Equilibrium Surface Tension Properties of Alcohol Ethoxylates¹

¹ Comparison of predicted and observed correlations of static surfactant properties with structural features of alcohol ethoxylates. Surfactant property data from Table 22; values followed by the same letter are not significantly different for that surfactant property. ² See Table 10 for full description of alcohol ethoxylates.

³ Data from Table 10.

⁴ Hydrophilic-lipophilic balance.

 5 EO = oxyethylene units; weight percent EO calculated from HLB using formula, weight % EO = 5 x HLB.

⁶ Minimum surface tension produced (at CMC). Note that increased surface tension values indicate decreased efficiency. Values in parenthesis are average values from Table 21.

⁷ Critical micelle concentration (in millimoles per liter).

⁸ Concentration to reduce surface tension of water by exactly 20 dynes/cm. ⁹ From Table 11.

As indicated in Table 31, the efficiency values among this set of alcohol ethoxylates are not significantly different although there is some indication of decreasing values with increasing EO content.

Table 32 shows the corresponding dynamic surface tension properties for the two sets of alcohol ethoxylates. The observed pattern of response to the two structural features is noted and compared to the predicted pattern.

Dynamic Surface Tension Properties of Alconor Ethoxylates								
Alcohol Ethoxylate ²	Average Alkyl Chain Length ³	HLB ^{3,4}	Weight % EO ⁵	Effective- ness (dynes/cm) ⁶	CMC (mM) ⁷	Efficiency (mM) ⁸		
A ₈₋₁₀ EO ₅	9.2	12.0	60.0	27.5	10.2	0.84 a		
A ₁₂₋₁₃ EO _{6.5}	12.5	12.1	60.5	36.6	16.6	1.36 a		
A ₁₂₋₁₄ EO _{7.0}	13.6	12.0	60.0	41.9	19.5	2.92 a		
			Observed Pattern	Decrease	Increase	Decrease		
			Predicted Pattern ⁹	Decrease	Increase	Decrease		
A ₁₂₋₁₃ EO ₃	12.5	7.9	39.5	53.8	19.2 a	>19.2		
A ₁₂₋₁₃ EO ₆₅	12.5	12.1	60.5	36.6	16.6 a	1.36 a		
A ₁₂ EO ₂₃	12.0	16.9	84.5	46.5	9.0	0.56 a		
12 25			Observed Pattern	?	Decrease	Increase		
			Predicted Pattern ⁹	Decrease	?	Increase as CMC decreases		

Table 32 oparties of Alcohol Ethovylates¹

¹ Comparison of predicted and observed correlations of dynamic surfactant properties with structural features of alcohol ethoxylates. Surfactant property data from Table 23; values followed by the same letter are not significantly ² See Table 10 for full description of alcohol ethoxylates. different for that surfactant property.

³ Data from Table 10.

⁴ Hydrophilic-lipophilic balance.

 5 EO = oxyethylene units; weight percent EO calculated from HLB using formula, weight % EO = 5 x HLB. ⁶ Minimum surface tension produced (at CMC). Note that increased surface tension values indicate decreased ⁷ Critical micelle concentration (in millimoles per liter). efficiency. ⁹ From Table 11.

⁸ Concentration to reduce surface tension of water by exactly 20 dynes/cm.

As shown in Table 32, dynamic surfactant effectiveness decreases with increasing alkyl chain length (with the weight % EO held constant). This is the pattern predicted by Myers (1991) based on the expectation that increasing the alkyl chain length would increase the time to reach equilibrium.

Increasing the alkyl chain length would be expected to increase dynamic CMC values, again because of increased time to reach equilibrium. As shown in Table 32, CMC values do increase with increasing alkyl chain length. Moreover, efficiency values would be expected to decrease with increasing CMC and that pattern is observed even though the efficiency values are not significantly different.

Considering the alcohol ethoxylates with constant alkyl chain length, increasing the weight % EO would be expected to decrease the dynamic surfactant effectiveness due to increased time to reach equilibrium. As indicated in Table 32, the observed pattern is more complex, suggesting maximum dynamic effectiveness (minimum surface tension value) at an intermediate weight % EO.

Dynamic CMC would be expected to increase with increasing weight % EO due to increased water solubility and increased time to reach equilibrium. In fact, dynamic CMC seems to decrease with increasing weight % EO. In confirmation of this unexpected pattern, dynamic efficiency increases, consistent with the inverse relationship between efficiency and CMC.

Surface Tension Measurement Correlations to Eye Irritation Potential

Fred Heitfeld (Appendix 8) has conducted an analysis for correlations between the eye irritation potential of the surfactant solutions and the equilibrium surface tension effectiveness (CONDEA Vista and U.S. Testing data), interfacial tension and contact angle. Mr Heitfeld plotted surface tension properties versus the maximum average scores (MAS) and days to clear (DTC) from the low volume eye test. He concluded that there was no apparent correlation between eye irritation potential and surface tension or interfacial tension. However, an apparent correlation was observed with contact angle. The correlation coefficients were 0.16 for MAS and 0.35 for DTC. These improved to 0.6 and 0.55, respectively, when the data for $A_{12-13}EO_3$ was deleted.

The data for contact angle, MAS and DTC are summarized in Table 33. Notice that Mr. Heitfeld was using the preliminary data on contact angle (values in parenthesis) for his analysis. In some case the final values are considerably different. The linear regression analysis curve for contact angle and MAS is shown in Figure 25, and for contact angle and DTC in Figure 26. The correlation coefficients for both lines are smaller than those reported by Mr. Heitfeld, apparently due to the use of the preliminary rather than the final data on contact angle. Neither line is statistically significant by the t-test. Deleting the data for $A_{12-13}EO_3$ (data point furthest to the left in both figures), will obviously improve the correlation coefficients but there is no justification for deleting the data on this surfactant. Moreover, the correlation of contact angle with other equilibrium surface tension properties suggests that it would be unlikely for a significant correlation to eye irritation potential to be observed just with contact angle.

Correlation coefficients from linear regression analysis of all of the surfactant properties vs. MAS are summarized in Table 34. There are no significant correlations to any of the equilibrium surface

tension properties. The only significant correlations are to the dynamic surface tension effectiveness at the two highest bubble rates, 1 and 4 bubbles per second. The fact that the correlation coefficients increase with the bubble speed suggests two conclusions: 1) it is the speed that the surfactant maximally reduces surface tension which correlates with the maximum average scores in the low volume eye test; and 2) even higher correlations might be achieved at faster bubble rates.

Nonionic Surfactant ²	MAS ³	DTC ³	Contact Angle ^{5, 6}
A ₁₂₋₁₃ EO ₃	0.0	0	23.0 (11.7)
Sorbitan trioleate-EO ₂₀	0.7	1	79.0 (85)
A ₁₂ EO ₂₃	1.3	1	87.0 (88.3)
A ₁₂₋₁₄ EO ₇	4.7	3	47.0 (46.3)
A ₁₂₋₁₃ EO _{6.5}	7.0	4	36.0 (33.7)
Cocamide DEA	7.3	1	67.0 (68.3)
$\begin{array}{c} \mathbf{A}_{12-16} - \\ \mathbf{glucose}_{1.6} \end{array}$	8.7	1	86.0 (74.3)
A ₈₋₁₀ EO ₅	11.3	4	32.0 (29.3)
Lauramine oxide (pH=7.0)	11.7	3	39.0 (35.0)
Lauramine oxide (pH=10.5)	13.0	1	53.0 (58.0)
Nonylphenol- EO ₉	13.3	4	56.0

Table 33	
Contact Angle Data Correlated with	Eve Test Scores ¹

¹ Summary of eye irritancy and contact angle data on nonionic surfactants to be examined for correlations. See Appendix 6 for contact angle data. ² See Table 10 for full description of nonionics.

³ Maximum average scores (MAS) and days to clear (DTC) from Table 16. Note that DTC is the longest period for the eyes of all rabbits exposed to a particular surfactant solution to clear. Consequently, it is different from the median days to clear shown in Table 16. 5 Units = degrees.

⁶ Numbers in parentheses are the values from the preliminary report from U.S. Testing Company, dated January 12, 1995 (Appendix 6). These are the values used by Fred Heitfeld in his analysis of the correlation between contact angle and MAS and DTC. In some cases these values are considerably different from the final values given in the Report of Test, February 1, 1995. Only values from the preliminary report which differ from those in the final report are shown.







R-square=.17, t=1.36, DF=9, p>.2

5

v	¥			r		
Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						-
Effectiveness ²	0.024	11				
Effectiveness ³	0.075	11				
Effectiveness ⁴	0.258	11	1.77	0.1		
Effectiveness: Average	0.136	11				
CMC ⁴	0.034	11				
Efficiency ⁴	0.07	11				
Interfacial Tension ³	4 x 10 ⁻⁶	11				
Contact Angle ³	0.038	11	0.60	>0.5		
DYNAMIC ⁴ :						
Effectiveness- slow ⁵	0.253	11				
Effectiveness- medium ⁵	0.531	11	3.19	<0.02	-0.428	23.6
Effectiveness- fastest ⁵	0.620	11	3.84	<0.01	-0.339	21.6
СМС	0.14	11				
Efficiency	0.0022	9				

Table 34Statistical Analysis Summary: Equilibrium Surface Tension vs. Eye Test Scores1

¹ Linear regression analysis of the maximum average score (MAS) in the low volume eye test of nonionic surfactants (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Except for contact angle (see text), significance was not calculated for R²-values smaller than 0.30. Slope of line and Y-intercept values are reported only for regression lines with significant ($p \le 0.05$) correlation coefficients.

² CONDEA Vista data. ³ U.S. Testing data. ⁵ Bubble speed -- see Table 24 for rates.

⁴ SynsaDyne data

.

The linear regression curves for the analyses of dynamic surface tension effectiveness (at the two highest bubble rates) versus the maximum average scores are shown in Figure 27 and Figure 28. Both curves show that MAS decreases with decreasing effectiveness (increasing surface tension at the CMC) under dynamic conditions.



In the SDA Phase III testing (Bagley et al., 1994), the logarithm transformation of the MAS data was used for the comparisons because better correlations were observed with the <u>in vitro</u> tests intended to predicted eye irritation potential. The logarithm transformation is performed by adding "1" to all values of any data set containing a zero value since the logarithm of zero is undefined. Since the MAS data for the nonionic surfactants (Table 19) does contain of value of zero, 1 (one) was added to all values before the logarithms were calculated. The correlation coefficients between the surface tension properties in Tables 21-25 and the logarithm of the MAS+1 values are shown in Table 35. Comparison of the correlation coefficients with those of Table 34 reveals that the correlation coefficients were generally not greatly affected by the logarithm transformation. Dynamic surface tension effectiveness remains the only surface tension property to show a significant correlation to the MAS values. Consequently, the logarithm transformation was unsuccessful in improved the correlations between MAS and the surface tension properties measured in these experiments.

Other data transformations were not examined because of the lack of justification for the transformations.



CONCLUSIONS

- 1. A review of the published and unpublished data on the eye irritation potential of nonionic surfactants revealed a number of uncertainties due to the concentrations tested (often undiluted, neat materials) versus the concentrations used in laundry and cleaning products, variations in the quality and quantity of the <u>in vivo</u> data, and inconsistency among some of the data. Consequently, there was a need for high quality eye irritation data on nonionic surfactants to provide a data base for the evaluation of non-animal (<u>in vitro</u>) tests for predicting eye irritation. The results of testing the nonionic surfactants described in this report provides this data.
- 2. Eleven nonionic surfactant solutions were prepared at a target concentration of 0.2 M. Errors in preparing two of the surfactant solutions ($A_{12}EO_{23}$, and sorbitan trioleate- EO_{20}) apparently had no effect on the observed eye irritation scores since both are reported to be very mild even when tested undiluted. Surfactant property measurements were corrected for the actual concentrations of each surfactant tested.
- 3. The eye irritation potential of each surfactant solution was determined in the low volume eye test (LVET). Maximum average scores (MAS) ranged from 0.0 to 13.3, and median days to clear ranged from 0 to 4 days. Consequently, all of the nonionic surfactant solutions tested have low potential for producing eye irritation.

Surface Tension Measurement	Correlation Coefficient (R ² -value)	Number of Data Points (n)	T-test Value (t)	Probability (p)	Slope of Line (b)	Y- intercept (a)
EQUILIBRIUM:						
Effectiveness ²	0.026	11				
Effectiveness ³	0.072	11				
Effectiveness ⁴	0.287	11				
Effectiveness: Average	0.146	11				
CMC ⁴	0.032	11				
Efficiency ⁴	0.103	11				
Interfacial Tension ³	4 x 10 ⁻⁶	11				
Contact Angle ³	0.038	11				
DYNAMIC ⁴ :						
Effectiveness	0.65	11	4.08	< 0.01	-0.0285	2.00
СМС	0.16	11				
Efficiency	0.009	9				

 Table 35

 Statistical Analysis Summary: Dynamic Surface Tension vs. Eye Test Scores¹

¹ Linear regression analysis of the logarithm of the MAS values (plus 1, see text) of nonionic surfactants (Table 19) versus surface tension measurements from Tables 21-25, using the equation Y = bX + a. Significance of correlation coefficient values was calculated using the t-test, where t = square root {DF x R² / (1 - R²)}, and DF (degrees of freedom) = n - 2. Except for contact angle (see text), significance was not calculated for R²-values smaller than 0.30. Slope of line and Y-intercept values are reported only for regression lines with significant (p \leq 0.05) correlation coefficients. ² CONDEA Vista data. ³ U.S. Testing data. ⁴ SynsaDyne data

4. The range of MAS results was narrower than expected. However, the results are reasonably consistent with previous testing on surfactant solutions conducted by the Non-animal Testing Subcommittee, and the relative order of the MAS results is consistent with the available literature on structurally similar nonionics.

5. Statistical analysis (ANOVA) revealed that the difference among the MAS scores for the surfactant solutions were highly significant (p<0.001). Further analysis of the scores using the paired t-test allowed the surfactant solutions to be split into two groups (of four each)

with significantly different MAS scores. The first group $(A_{12-13}EO_3, \text{ sorbitan trioleate-}EO_{20}, (A_{12}EO_{23}, \text{ and } A_{12-14}EO_7)$ had MAS scores ranging from 0.0 to 4.3 while the second group $(A_{8-10}EO_5, \text{ lauramine oxide (pH=7.0)}, \text{ lauramine oxide (pH=10.5)} \text{ and nonylphenol-}EO_9)$ had MAS scores ranging from 11.3 to 13.3. The remaining three surfactants $(A_{12-13}EO_{6.5}, \text{ cocamide DEA and } A_{12-16} - \text{ glucose}_{1.6})$ had intermediate MAS scores which were not significantly different from the first two groups.

- 6. An analysis of the unpublished eye irritation data (Draize test) on alcohol ethoxylates suggested that the MAS scores of these materials correlate with a structural property, the hydrophilic-lipophilic balance (HLB). This hypothesis was confirmed with the results observed for the five alcohol ethoxylates among the nonionic surfactants tested. The three alcohol ethoxylates with HLBs of 12 ($A_{12-13}EO_{6.5}$, $A_{12-14}EO_7$ and $A_{8-10}EO_5$) had MAS scores higher than the two alcohol ethoxylates with either a lower HLB ($A_{12-13}EO_3$) or a higher HLB ($A_{12}EO_{23}$). Among the three alcohol ethoxylates with an HLB of 12, the MAS scores increase with shorter alcohol chain lengths.
- 7. Among the nonionic surfactants tested, no correlations to pH or to structural properties were noted.
- 8. The eleven surfactant solutions were also tested for their surfactant properties: equilibrium surface tension effectiveness, critical micelle concentration (CMC) and efficiency, interfacial tension, contact angle, dynamic surface tension effectiveness, CMC and efficiency.
- 9. Three laboratories were used to measure equilibrium effectiveness. The results from the three labs were very similar for nearly all the nonionic surfactants tested, confirming the validity of the different equipment and test procedures used. The one exception was for the sorbitan trioleate-EO₂₀ solution which was reported by two of the laboratories to be viscous. Apparently, the viscosity of this solution interfered with the measurement of equilibrium effectiveness, reducing the agreement among the laboratories on the values for this material.
- 10. A similar pattern of response with the surfactant solutions was observed for equilibrium effectiveness, interfacial tension and contact angle, suggesting that these properties are related, at least for nonionic surfactants. Based on the magnitude of the response and the number of significant correlations to structural properties, the most sensitive surfactant property among these three seems to be contact angle while interfacial tension seems to be the least sensitive.
- 11. The pattern of response of the surfactants to equilibrium and dynamic conditions differ substantially, indicating that the speed (in the tenths of a second time range) with which the surfactant molecules move to the interface and reduce the surface tension differ among the nonionics tested.
- 12. Physical and structural properties of nonionic surfactants were considered to determine if any

of these predicted surface tension properties. The HLB, and to a lesser extent the number of EO units per molecule, was negatively correlated to the CMC values, especially under more dynamic conditions. The number of EO units, and to a lesser extent the HLB, was negatively correlated to contact angle and equilibrium effectiveness, but not to interfacial tension. Unfortunately, none of the physical or structural properties examined was correlated to dynamic effectiveness, the one surfactant property which significantly predict eye irritation potential.

- 13. Considering the structure of the alcohol ethoxylates tested, the pattern of response for equilibrium and dynamic effectiveness was consistent with the pattern reported in the literature. Some exceptions were noted to the patterns predicted for CMC and efficiency, indicating that our understanding of how structural properties affect surface tension properties is far from complete.
- 14. Previous work by the Subcommittee suggesting that contact angle predicted the MAS scores was found to be based on preliminary data for contact angle. Use of the final values from the contract laboratory showed no significant correlation between contact angle and MAS.
- 15. A significant correlation between MAS results and dynamic surface tension effectiveness property was demonstrated. Statistically significant correlations (p<0.02) were observed at the two highest bubble rates tested (1 and 4 bubbles per second), the most dynamic conditions tested. The correlation coefficients for this analysis ranged from 0.53 to 0.62, indicating that 53 to 62% of the variation in the MAS results is accounted for by the variation in the dynamic surface tension effectiveness. The fact that the correlation coefficients increase in going to the highest bubble rate tested suggests that surface tension effectiveness at higher bubble rates should be investigated to determine if higher correlations are possible.
- 16. No other significant correlations between MAS and surface tension properties were found, even after using the logarithmic transformation of the MAS scores, which improved correlations between tests in previous Subcommittee research.
- 17. The fact that dynamic, but not equilibrium, surface tension effectiveness correlates to MAS suggests that the ability of a surfactant to reduce surface tension in the tenths of a second time frame is a critical parameter for producing eye irritation. Note that the bubble rates at which the correlations were observed (1 and 4 bubbles per second) allow less than one second for the surfactant to move to the interface and reduce the surface tension.

REFERENCES

Bagley, D., K. A. Booman, L. H. Bruner, P. L. Casterton, J. Demetrulias, J. E. Heinze, J. D. Innis, W. C. McCormick, III, D. J. Neun, A. S. Rothenstein, R. I. Sedlak (1994). The SDA alternatives program. Phase III: Comparison of in vitro data with animal eye irritation data on solvents, surfactants, oxidizing agents, and prototype cleaning products. *J. Toxicol.--Cut. & Ocular Toxicol.* 13: 127-155.

Benke, G. M., N. M. Brown, M. J. Walsh and R. B. Drotman (1977). Safety testing of alkyl polyethoxylate nonionic surfactants. I. Acute effects. *Fd. Cosmet. Toxicol.*, 15: 309-318.

Cosmetic Ingredient Review (1986). Final report on the safety assessment of cocamide DEA, lauramine DEA, linoleamide DEA and oleamide DEA. J. Am. Coll. Toxicol. 5: 415-454.

Cox, M. F. (1989). Effect of alkyl carbon chain length and ethylene oxide content on the performance of linear alcohol ethoxylate. J. Am. Oil Chem. Soc. 66: 367-374.

Cox, M. F. (1990). The effect of "peaking" the ethylene oxide distribution on the performance of alcohol ethoxylates and ether sulfates. *J. Am. Oil Chem. Soc.* 67: 599-604.

CTFA (1977). *Cosmetic Ingredient Dictionary*. Second Edition (N. F. Estrin, ed.), The Cosmetic Toiletry and Fragrance Association, Inc., Washington, DC 20005.

Draize, J. H., and E. A. Kelley (1952). Toxicity to eye mucosa of certain cosmetic preparations containing surface-active agents. *Proc. Sci. Sect. Toilet Goods Assoc.*, 17: 1-4.

ECETOC (1992). Technical report no. 48. Eye irritation: Reference chemicals data bank. European Center for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

Feldman, J. B., P. DeLong, and C. P. Brown (1948). Practical application of surface-active drugs in ophthalmology. *Arch Ophthalmol.*, 40: 668-679.

Gingell, R. (1994). Shell Oil Co., personal communication.

Grant, W. M. (1986). Toxicology of the Eye, third edition, Charles C. Thomas, Springfield, IL.

Griffin, W. C. (1949). Classification of surface-active agents by "HLB". J. Soc. Cosmet. Chem. 1: 311-326.

Griffin, W. C. (1954). Calculation of hydrophile-lipophile balance values of non-ionic surfactants. *J. Soc. Cosmet. Chem.* 5: 249-256.

Griffith, J. F., G. A. Nixon, R. D. Bruce, P. J. Reer, and E. A. Bannan (1980). Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. *Toxicol. Appl. Pharmacol.*, 55: 501-513.

Hagiwara, H., and S. Sugiwia (1952). The use of castor-oil and Tween 80 as an ophthalmic base (title translated by Grant, 1989, page 754). *Acta Soc. Ophthalmol. Jpn.* 57: 1-5.

Hazleton, L. W. (1952). Relation of surfactant active properties to irritation of the rabbit eye. *Proc. Sci. Sect. Toilet Goods Assoc.*, 17: 5-9.

Kennah, II, H. E., S. Hignet, P. E. Laux, J. D. Dorko, and C. S. Barrow (1989). An objective procedure for quantitating eye irritation based on change of corneal thickness. *Fund. Appl. Toxicol.* 12: 258-268.

McCormick, W. (1993). Clorox Corp., personal communication.

Myers, D. (1991). Surfaces, Interfaces, and Colloids. Principles and Applications. VCH Publishers, New York, N.Y.

Neun, D. J. (1993). Effects of alkalinity of the eye irritation potential of solutions prepared at a single pH. J. Toxicol.--Cut. & Ocular Toxicol. 12:227-231.

NIOSH (1993). *Registry of Toxic Effects of Chemical Substances*. National Institute of Occupational Safety and Health, Washington, D.C., on-line.

North-Root, H., F. Yackovich, J. Demetrulias, M. Gacula, Jr., and J. E. Heinze (1982). Evaluation of an in vitro cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicol. Lett.* 14: 207-212.

Roberts, D. W. (1991). QSAR issues in aquatic toxicity of surfactants. *Sci. Total Environ.* 109/110: 557-568.

Schott, H. (1996). Effects of electrolytes and protein denaturants on nonionic surfactants. *Tenside Surf. Det.*, 33: 457-463.

Smith, D. (1994). CONDEA Vista Co., personal communication.

Smith, G. (1997). Henkel Corp., personal communication.

Treon, J. F. (1965). Physiological properties of selected non-ionic surfactants. Soap Perfum. Cosmet., 38: 47-54.

Appendix 1 Eye Irritation Potential of Alcohol Ethoxylates: Summary Reports of Unpublished Studies by Subcommittee Member Companies

- 1) Memo from J.D. Innis (Procter & Gamble Company), dated February 10, 1992 (two pages).
- 18) Memo from R. Gingell (Shell Oil), dated March 9, 1992 (two pages).
- 19) Table: "CONDEA Vista Comprehensive Data," prepared by J.E. Heinze, December 13, 1996 (one page).
- 20) Table: "Calculation of Weight Percent EO from Moles EO," prepared by J.E. Heinze, December 13, 1996 (one page).

Appendix 2

Nonionic Surfactants Selected by the Subcommittee for Contract Testing: Information Provided by Suppliers and the *CTFA Cosmetic Ingredient Dictionary (1977)*

- 1) Letter from R. Gingell (Shell Oil) with five pages from the "Bubbles" product brochure on NEODOL alcohol ethoxylates, Material Safety Data Sheets (MSDSs), Environmental Data sheets and Test Substance Identification sheets (17 pages).
- 2) Technical Data brochures, MSDSs and Certificates of Analysis from CONDEA Vista on ALFONIC alcohol ethoxylates (33 pages).
- 3) Product Information Bulletin, MSDS and Certificate of Analysis from ICI Americas on BRIJ-35 (six pages).
- 4) Product information bulletin, MSDS and Certificate of Analysis from Union Carbide on TRITON N-101 (11 pages).
- 5) Product information bulletin and MSDS from ICI Americas on TWEEN 85 (six pages).
- 6) Technical Data Sheet, MSDS and Certificate Request from Henkel on GLUCOPON 625CS (seven pages).
- 7) Two pages from Product Guide, MSDS, Specification Sheet and Analysis Report from Croda on INCROMINE OXIDE L (eight pages).
- 8) Product information sheet, MSDS and Certificate Request from Henkel on STANDAMID KD (seven pages).
- 9) Selected pages from the CTFA Cosmetic Ingredient Dictionary (1977) (eight pages).

Appendix 3 Hazleton Wisconsin, Inc.: Low Volume Eye Test Final Report

.

Appendix 4 Hazleton Wisconsin, Inc.: Low Volume Eye Test Raw Data

Appendix 5 CONDEA Vista Company: Surface Tension Methods and Data

- 1) Letter from D. Smith to J. Al-Atrash, dated October 28, 1994 (two pages).
- 2) Letter from D. Smith to J.E. Heinze, dated March 11, 1997 (one page).

Appendix 6 United States Testing Company: Surface Tension, Interfacial Tension and Contact Angle Methods and Data

- 1) Preliminary Report, dated January 12, 1994 (two pages).
- 2) Final Report, dated February 1, 1994 (four pages).
- 3) ASTM D 1331 89, "Standard Test Method for Surface and Interfacial Tension of Solutions of Surface-Active Agents (three pages).
- 4) ASTM D 724 94, "Standard Test Method for Surface Wettability of Paper (Angle-of-Contact Method) (three pages).
- 5) Fax from L. Van Savage to J. Heinze, dated 3/21/97 (one page).

Appendix 7 SensaDyne Instrument Division: Dynamic Surface Tension Methods and Data

- 1) Report: "Physical & Chemical Properties of Nonionic Surfactants, Surface Tension Study" - HWI# 6310-105.
- 2) Fax from T.C. Christensen to J. Al-Atrash, dated March 2, 1995 (three pages).
- 3) Fax from T.C. Christensen to J. Al-Atrash, dated March 21, 1995 (15 pages).
- 4) Fax from T.C. Christensen to J.E. Heinze, dated March 11, 1996 (one page).
- 5) Product brochure: "Surface Tensiomters" (eight pages).
- 6) Product brochure: "PC9000" (one page).
- 7) Presentation: S.M. Hosseini, "Dynamic Surface Property Measurement of Aqueous Surfactant Solutions," American Chemical Society, Northeast Regional Meeting, June 1992 (22 pages).
- 8) Presentation: V.P. Janule, "Three Dimensional Characterization of Active Surfactants," The Fine Particle Society, 23rd Annual Meeting, July 1992 (12 pages).

Appendix 8 Statistical Analysis

- 1) Letter from K. A. Booman to R. Sedlak, dated November 3, 1994 (five pages).
- 2) Fax from J. Heinze to J. Al-Atrash, dated March 16, 1995 (four pages).
- 3) Fax from J. Al-Atrash to Nonionic Surfactant Task Force, dated October 25, 1995 (11 pages).
- 4) Computer print out: "Stat-Packets Statistical Analysis Package: Analysis of Variance" (four pages).